The Journal of Parasitology

Volume 37 JUNE, 1951 Number 3

THE LIFE HISTORY OF *ALLOCREADIUM ICTALURI* PEARSE, 1924 (TREMATODA: DIGENEA)[1]

PHILIP G. SEITNER[2]

Department of Biological Sciences, Purdue University, Lafayette, Indiana

INTRODUCTION

Although the taxonomy of the digenetic trematodes has been based largely on adult morphology, life history studies have contributed much toward a natural classification. In many instances, they have confirmed taxonomic groups established when few life histories were known. In others, however, they have indicated that certain families based on similarity of adult structure are not natural groups of closely related trematodes. Furthermore, life history studies have demonstrated rather clearly a close relationship between certain families, e.g., the HETEROPHYIDAE and OPISTHORCHIIDAE, the PLAGIORCHIIDAE and the MICROPHALLIDAE, and the ECHINOSTOMIDAE and PSILOSTOMIDAE. Some investigators have utilized the superfamily concept to bring such related families together. As a result, there have been proposed the superfamilies OPISTHORCHIOIDEA, PLAGIORCHIOIDEA, ECHINOSTOMOIDEA and several others which, in the light of present knowledge, appear to be natural groups.

There is still a dearth of information concerning life histories, especially in the large group of trematodes collectively known as the allocreadiids. These flukes, almost exclusively parasites of fishes, have similar adult stages and have been allocated to various suprageneric categories. Of these, the family ALLOCREADIIDAE was the first to be proposed. More recent studies of adult morphology and a few scattered life histories led first to the proposal of allocreadiid subfamilies and then to the elevation of these to family rank with or without implication as to superfamily relationships. As a result, the confusion in regard to these trematodes is probably unequalled in any other large group of the DIGENEA and the basis for a natural classification still is not evident. It was for this reason that the present investigation was undertaken. It is the first life cycle of a species of *Allocreadium* to be demonstrated experimentally, and since the genus is the type of the family, it is hoped that this study will be a contribution to the problem of affinities within the group.

Received for publication, October 2, 1950.

[1] Based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Purdue University, Feb. 1951. This study was made under the direction of Professor R. M. Cable whose suggestions, criticism, and generous assistance have been invaluable.

[2] Present address, Dept. of Zoology, Louisiana State University, Baton Rouge, La.

Although certain historical material is deferred to the discussion, a brief review of the genus *Allocreadium* is desirable as a background for the writer's observations. In breaking up the old genus *Distomum,* Looss (1899) erected the genus *Creadium* with the following diagnosis (in translation): "Worms of sub-medium size with a thick body almost cylindrical in the contracted condition, rounded posteriorly, the anterior end attenuated in a slender, very active neck region; well-developed suckers, smooth cuticle. Intestinal tract with well-developed pharynx, long esophagus (in a contracted neck, bent S-shaped and, therefore, appearing rather short), and long intestinal ceca. Genital opening in the vicinity of the intestinal bifurcation. Cirrus pouch large, sac-shaped, with well-developed cirrus. Testes large and spherical, median, close behind one another in the posterior portion of the body. Ovary similarly large, displaced laterally. Seminal receptacle and Laurer's canal present, the former voluminous, pear-shaped. Vitellaria very abundant, confluent behind the testes and filling the entire lateral region of the body. Uterus short, describing several loops between the anterior testes and the ventral sucker. Eggs in a row, relatively large (0.06–0.09 mm.), and with feebly-colored shell. The first stages of development appear to occur in traversing the uterus. Parasites of (fresh-water?) fish. Type: *Creadium isoporum* Looss"; includes also *C. angusticolle* Hausmann, 1896.

It was brought to Looss' attention that the name *Creadium* was preoccupied and in 1900 he proposed the substitute name, *Allocreadium* ("another Creadium"). In defining the genus, Looss (1899) had suggested that possibly some worms described by Stossich might be closely related to *Creadium isoporum* and *C. angusticolle.* The following year, Stossich (1900) placed *Distomum obovatum* Molin, 1885, and *D. album* Stossich, 1886, in the genus *Allocreadium* and described two additional species, *A. pegorchis* and *A. asymphyloporum.*

Odhner (1901) redescribed several worms that were in agreement with Looss' definition of the genus. In addition to *Allocreadium isoporum* and *A. angusticolle,* Odhner included the following species in *Allocreadium: A. fasciatum* (Rudolphi, 1819) (= *Distoma fasciatum*); *A. sinuatum* (Rudolphi, 1819) (= *Distoma sinuatum*); *A. labri* (Stossich, 1886) (= *Distomum labri*); *A. genu* (Rudolphi, 1819) (= *Distoma genu*); *A. commune* (Olsson, 1868) (= *Distoma commune*); *A. tumidulum* (Rudolphi, 1819) (= *Distoma tumidulum*); *A. transversale* (Rudolphi, 1802 (= *Fasciola transversalis*); *A. atomon* (Rudolphi, 1802) (= *Fasciola atomon*); and *A. labracis* (Dujardin, 1845) (= *Distoma labracis*).

In the same year, Looss (1901) described *Distomum alacre* which Stossich later (1905) included in *Allocreadium.*

After studying a worm which he identified as *Distoma pulchella* Rudolphi, 1819, Odhner (1902) concluded that it and *Allocreadium labri* were synonymous and, due to some anatomical peculiarities (including eggs with polar filaments), he erected the genus *Helicometra,* with *H. pulchella* as type and including *Allocreadium fasciatum* and *A. sinuatum.* Looss (1902) erected the subfamily ALLOCREADIINAE to include the genera *Allocreadium* and *Helicometra* and two years later, Stossich (1904) added a third genus, *Lepocreadium,* with *L. album* (Stossich, 1886) as type and including *A. pegorchis* (Stossich, 1900).

Stossich (1904) elevated the ALLOCREADIINAE to the rank of family, ALLOCREADIIDAE; in 1905 he described *A. dubium* and transferred *Distomum alacre* Looss,

1901, *D. characis* Stossich, 1886, *D. mormyri* Stossich, 1885, and *D. umbrinae* Stossich, 1885, to the genus *Allocreadium.* In 1906, Odhner erected the genus *Podocotyle* to include *P. atomon* (Rudolphi, 1802) which he had previously assigned to the genus *Allocreadium.* He included this genus in the ALLOCREADIINAE and proposed a new subfamily, the LEPOCREADIINAE, for the genus *Lepocreadium.*

In a paper concerning allocreadiine worms, Nicoll (1909a) proposed three new genera: *Cainocreadium* for *Allocreadium labracis* (Dujardin, 1845); *Peracreadium* with *P. genu* (Rudolphi, 1819) as type and including *P. commune* (Olsson, 1868); and *Lebouria* with *L. idonea* Nicoll, 1909, as type and including *L. tumidulum* (Rudolphi, 1819) and *L. obducta* Nicoll, 1909. In the same paper, Nicoll proposed the group STEPHANOPHIALINAE, which was later to be included in the family ALLOCREADIIDAE. Nicoll also suggested that three species, referred to *Allocreadium* by Stossich (1900 and 1904), viz., *Distomum obovatum* Molin, 1885, *D. umbrinae* Stossich, 1885 and *D. mormyri* Stossich, 1885, did not belong in the genus *Allocreadium,* but, because of mutual resemblance, perhaps represented a distinct genus.

In another paper the same year, Nicoll (1909b) apparently transferred *Allocreadium alacre* (Looss) to the genus *Lebouria,* although he referred to the species as "*Lebouria alacris* sp. n.". He later (1910) had occasion to compare this form with specimens which more nearly agreed with Looss' original description of *Distomum alacre* and concluded that the two were distinct species. He accordingly named the first *L. varia,* and transferred both to the genus *Lebouria.*

In a review of the genus *Allocreadium,* Wallin (1909) described one species, *A. lobatum,* and proposed the name *A. colligatum* for a trematode previously identified by Stossich as *Distoma fasciatum* Rudolphi, but considered by Wallin to be a distinct species. He transferred to the genus *Allocreadium D. pallens* and *D. mormyri,* apparently unaware that Stossich had already made this disposition of the latter species. Of the species hitherto ascribed to the genus *Allocreadium,* Wallin did not include in his key *A. dubium* Stossich, 1905, *A. alacre* Looss, 1901, *A. characis* (Stossich, 1886) and *A. umbrinae* (Stossich, 1885). Of these, he discussed only *A. dubium* as a doubtful species and apparently overlooked the others. Wallin also made no note of the fact that four of the species which he included in *Allocreadium* had been previously transferred to other genera; these were *A. labracis* and *A. genu,* transferred to *Cainocreadium* and *Peracreadium* respectively; *A. atomon* to *Podocotyle;* and *A. fasciatum* to *Helicometra* (*vide supra*).

Since 1910, several new species have been placed in the genus *Allocreadium* and two of the earlier ones, *A. colligatum* and *A. angusticolle,* have been removed to *Helicometra* by Nicoll (1915) and to *Plagioporus* by Dobrovolny (1939) respectively. More recently, Mathias (1937) has reported briefly on the life cycle of the latter species and Dollfus (1949) has transferred it to the genus *Coitocaecum.*

Thus it is seen that although a number of species has been allocated to the genus *Allocreadium,* many have been transferred from it. Although it is by no means certain that this disposition is the correct one for all of them, the following species are included in the genus:

Allocreadium annandalei Southwell, 1913; *A. asymphyloporum* Stossich, 1900; *A. boelosomi* Pearse, 1924; *A. dubium* Stossich, 1905; *A. fowleri* Leiper and Atkinson, 1914; *A. handiai* Pande, 1937; *A. hasu* Ozaki, 1926; *A. ictaluri* Pearse, 1924; *A. isoporum* (Looss, 1894); *A. japonicum* Ozaki, 1926; *A. kosia* Pande, 1938;

A. lobatum Wallin, 1909; *A. madaseri* Pande, 1938; *A. mormyri* (Stossich, 1885); *A. nicolli* Pande, 1938; *A. obovatum* (Molin, 1858); *A. oncorhynchi* Eguchi, 1931; *A. pallens* (Rudolphi, 1819); *A. polymorphum* Layman, 1933; *A. pseudotritoni* Rankin, 1937; *A. schizothoracis* Pande, 1938; *A. transversale* (Rudolphi, 1802); and *A. umbrinae* (Stossich, 1885).

Previous to this study, no life history of a species of *Allocreadium* has been demonstrated experimentally, although Looss (1894) described stages of what he considered to be *A. isoporum* on the basis of morphological and ecological observations. He described as the larva of *A. isoporum* a cercaria developing in the liver of the sphaeriid bivalves, *Cyclas cornea* and *C. rivicola.* His evidence was based on morphological comparisons, specifically the presence of eyespots in the larva, their remnants in the adult, and similarities in the excretory system and genital primordia. His figure of the larva depicts an ophthalmoxiphidiocercaria with an unusually large, unadorned tail. Although Looss did not find the metacercaria, Dollfus (1949) has recently reported its occurrence in the visceral mass of the same species of sphaeriids serving as the first intermediate host. He recovered metacercariae from individuals harboring larval generations of the trematode as well as those otherwise uninfected. There is only one other reference to a stage in the life cycle of a species of *Allocreadium.* This concerns a metacercaria found by Clark and Wilson (1911) in mussels from the Maumee and Kankakee Rivers. Hopkins (1934a) identified this metacercaria as that of *Allocreadium ictaluri,* the species whose life cycle is the subject of the present study.

OBSERVATIONS

Experimental Determination of the Life Cycle

The present study began with the discovery of metacercariae in the edge of the mantle of unionid bivalves collected from the Tippecanoe River, Indiana. Later, it was found that mussels in the Wabash River also were infected. Since the encysted parasites were always present in large numbers, an abundance of material was assured. The next step was an attempt to discover the adult worm both by feeding metacercariae to a variety of animals known to eat clams, and which for that reason might serve as the definitive host, and by examining such animals for natural infections. Various species of turtles taken from the Tippecanoe River yielded no parasites that seemed likely to be the adult worm sought and metacercariae fed to turtles were killed in passing through the alimentary tract. Muskrats were next investigated, since they lived along the river and their consumption of mussels was indicated by mounds of shells. The only trematode found to occur in this animal was an echinostome. Laboratory rats were fed metacercariae which either failed to excyst or were killed in passage through the intestine. Newly hatched chicks were then fed infected mantle tissue with negative results. At the time, it did not seem that a fish would be likely to serve as the definitive host, considering the difficulty of opening or crushing infected bivalves. Since other feeding experiments had been negative, metacercariae were fed to a small catfish which had been brought into the laboratory incidental to some other work. Empty cysts were found in the feces and when the fish was killed later, young worms were recovered from the intestine. These were identified as immature adults of *A. ictaluri* which was subsequently found in naturally infected catfish taken from the Wabash River.

To confirm and extend the above results, additional feeding experiments were made. Since mussels from Eel River at North Manchester, Indiana, had been examined and found free of metacercariae, six catfish (*Amieurus* sp.) were taken at that site during mid-winter. Four that were killed in being caught were examined and found to be free of *Allocreadium ictaluri.* After over a month's conditioning to an aquarium, one of two surviving fish was fed metacercariae and a few days thereafter was killed and examined for trematodes. Nine months later, the second fish was utilized in the same manner. In both cases, considerable numbers of small, immature *A. ictaluri* only were found. In view of the conditions of the experiment, it seems certain that the worms recovered were from the metacercariae the fish had eaten.

It has been observed that the metacercariae remain alive and active even after the molluscan host has died and reached an advanced state of decomposition. Mussels dying naturally in the river are most often found with the valves lying open and the soft parts exposed. Catfish in the laboratory were observed tearing the flesh from the shells of opened mussels and showed a preference for putrid tissue. It would seem that in the case of *A. ictaluri,* the metacercaria may remain in the mussel for a period of several years, depending upon the natural death of that host for transfer to the definitive host.

Although fish might have difficulty in getting at the flesh of large mussels such as those utilized in all the feeding experiments in this study, it has been observed that the cercaria will penetrate and develop in sphaeriids. These are very small and could readily be ingested entire by the definitive host as could young individuals of the large Unionidae.

Since the metacercariae possessed scattered eyespot pigment, the cercaria was obviously biocellate. Such larvae known to occur in the vicinity included pleurolophocercous, monostome and amphistome species from gastropods and ophthalmoxiphidiocercariae from sphaeriid bivalves. On the basis of known life cycles, none of these could be considered to be the larva of *A. ictaluri.* Thus was eliminated from consideration all except a cercaria imperfectly known from a survey made by another investigator some years before. This undescribed larva, which developed in *Pleurocera acuta,* possessed eyespots and a simple tail, but lacked a stylet. Since it was the only known one in the locality that seemed at all likely to be the larva of *A. ictaluri,* an intensive effort was made to find it again. First, snails were collected from the Tippecanoe River, where the bivalves were heavily infected with metacercariae. However, examination of many thousands of *P. acuta* and *Goniobasis livescens* failed to reveal the larva in question. Snails were then collected about two miles below the confluence of the Wabash and Tippecanoe Rivers. After only a few hundred of these had been examined, a snail shedding the cercaria sought was found and several additional ones were obtained later from the same locality. The only explanation for the absence of infected snails in about a quarter-mile extent of the Tippecanoe River in which bivalves are heavily infected with metacercariae seems to be that snails shedding cercariae are spotty in their distribution. The larvae must drift with the current a considerable distance before they become negatively phototropic and swim downward to enter the bivalve second intermediate host.

Penetration and encystment of the cercaria were first demonstrated by placing

four infected snails in a deep dish containing two small mussels from Eel River where, as already mentioned, no infected molluscs could be found. A layer of clean sand was provided to encourage activity and the water was left undisturbed until the snails were removed several days later. After two weeks, the mussels were sacrificed and a number of young metacercariae was found in the soft inner tissues at the edge of the mantle. These measured approximately 0.13 mm in diameter. Little, if any, of the host tissue proliferation, characteristic of old, natural infections, had yet occurred, although the body of the worm appeared more granular and opaque than in the cercaria and there was a slight dispersion of the eyespot pigment.

Several difficulties were encountered which prevented experimental infection of snails with miracidia and subsequent observations on the early larval stages in that host. In the first place, the supply of catfish was limited to some extent, heavy infections with *A. ictaluri* were infrequent, and in an infected fish it was very unusual to find more than three or four sexually mature worms. Furthermore, this species contains only a few eggs in the uterus; even in old individuals, few of which were obtained, scarcely more than a hundred eggs were present. Hence, large numbers were never available.

At first, eggs obtained from the uteri of worms were placed in shallow dishes of water aerated with a fine stream of air and examined daily. Few handled in this manner developed until eyespots appeared; most of those that became ciliated and motile, died without hatching. Each of three young snails was exposed to a single miracidium which disappeared, but was not observed to enter the snail. After exposure, the snails were kept in dishes of water with a bottom layer of sand and mud and to which soft algae from an outdoor pool was added periodically. Two of these snails died within a month and the third was killed shortly thereafter. On examination, none of these gave evidence of infection with larval trematodes. The few other miracidia that hatched at that time were used to study their morphology.

Recently, renewed attempts to obtain miracidia have been more successful. It has been found that eggs removed from the uteri of worms, placed in dishes of water and let stand without aeration developed with far better results than before. In this manner, numerous miracidia were obtained on one occasion. Many were used for morphological studies, either living, fixed and stained, or silver impregnated to delineate epidermal cell boundaries. Several others were used to expose snails to infection. Sufficient time has not lapsed to determine whether this experiment has been successful.

Stages in the Life Cycle

(All Measurements in Millimeters)

The Adult (Fig. 1)

Allocreadium ictaluri occurs in the intestine of various species of catfish. Pearse (1924) first described it from the channel catfish, *Ictalurus punctatus* and Mueller and Van Cleave (1932) later reported it from *Ameiurus nebulosus.* In the present study, the species has been found also in the blue catfish, *I. furcatus,* although most use has been made of specimens from the channel catfish.

Mueller and Van Cleave (1932) named the worm *A. halli,* because of differences between their specimens and *A. ictaluri* as described by Pearse. These differences

were in respect to form of the body, size of the eggs and shape of the testes and intestinal crura, based largely on Pearse's figure of *A. ictaluri*. Later, Van Cleave and Mueller (1934) examined Pearse's specimens and reduced *A. halli* to synonomy with *A. ictaluri*. The present study supports this view and indicates that discrepancies in the existing descriptions may be attributed to differences in age and handling of material. Morphology of the adult *A. ictaluri* is shown in Fig. 1 and a detailed description of its structure need not be repeated here. It is desirable to record certain observations, however, since they extend and clarify existing descriptions, particularly of the excretory system.

Flukes from naturally infected catfish vary from small, immature distomes less than 1.0 long to sexually mature individuals over 4.0 in length. This variation is often found in worms from a single host. Young specimens which were smaller than the largest metacercariae found in mussels, were often recovered; this indicates that the metacercaria becomes infective before attaining maximum size in the second intermediate host.

The largest of the writer's specimens of *A. ictaluri* was taken from a channel catfish from the Wabash River and measured 4.3 in length and 1.0 in width at the acetabular level, while relaxed and under very slight cover glass pressure. Pearse (1924) recorded a length of 5.9 and a width of 1.85 while Mueller and Van Cleave gave 3.5 as the maximum length. The writer has never observed in living or promptly fixed material the inflated intestinal ceca described by Mueller and Van Cleave; instead, the caeca were found to correspond more closely to Pearse's representation of them. However, in other respects (presence of esophagus and smooth contour of testes), the author's observations are more in agreement with those of Mueller and Van Cleave.

The excretory vesicle is thin-walled and conspicuous in the living worm. It empties posteriorly through a short, muscular channel leading to the terminal excretory pore. A prominent sphincter is present. The vesicle extends to a point slightly beyond the anterior margin of the anterior testis where the main excretory canals enter it separately. From the vesicle, the main canals extend anteriorly in a tortuous path to the pharyngeal level. There each turns abruptly and follows a posterior course, ventral and roughly parallel to its anterior path. At the level of the cirrus sac, each main canal is formed by the junction of two collecting ducts, one extending anteriorly, the other posteriorly.

Three groups of flame cells join each anterior and posterior collecting duct so that there are six groups on each side of the body. The three joining each anterior duct lie anterior to the mid-acetabular level, while the remaining three on each side are posterior to that level. For convenience in description, the flame cell groups on each side are numbered one to six from anterior to posterior.

The first group of flame cells is at the level of the posterior edge of the oral sucker and functions in the oral and pharyngeal region. A long collecting tubule extends posteriorly from this group to join a short collecting tubule from the second group whose capillaries converge opposite the anterior end of the cirrus sac. Flame cells associated with the second group lie as far cephalad as the pharyngeal level and as far posteriorly as the level of the anterior edge of the acetabulum. The third group converges at a point slightly anterior to the mid-acetabular level, serving the thickest part of the worm. The collecting tubule from this group extends anteriorly

to join the tubule from the first and second groups, thus forming the anterior collecting duct.

The capillaries of the posterior-most or sixth flame cell group converge opposite the posterior testis and the flame cells are distributed from its anterior edge to the posterior end of the body. A long tubule leads from this group to the collecting tubule of the next, i.e., fifth, group, situated slightly anterior to the anterior testis. The fourth group lies opposite the posterior edge of the acetabulum and its collecting tubule unites with the tubule draining the posterior two groups, forming the posterior collecting duct. This duct extends anteriorly to join the anterior collecting duct, forming the main excretory canal as described above.

The flame cells of *A. ictaluri* are very similar to those described by Looss (1894) for *A. isoporum;* in the mature worm, they become large and flat, although funnel-shaped in outline, and hence are quite different from the flame cells of the immature stages where they have the usual appearance. The capillary tubules are pigmented in the mature worm and appear as distinct, granular, dark lines. This pigmentation does not extend as far as the flame cells nor is it found in the larger collecting ducts. The flame cell capillaries of a group do not unite in a manner suggestive of palmate or dichotomous branching, but instead form an irregular pattern best described as a series of consecutive tributaries to the origin of a collecting tubule which leads away from the group to join that of the adjacent group.

The flame cells are easily seen in large worms. It is much more difficult to observe all the flame cells and their capillary pattern in old than in young specimens, because large ones are so thick that cover glass pressure will not flatten them sufficiently to permit observation of the capillaries on both dorsal and ventral surfaces of the same preparation.

In young adults, the flame cell number and the capillary pattern within single groups were determined accurately. However, the flame cells are so numerous in this species that the arrangement of their capillaries could not be determined for all groups in a single specimen before its death. In observing a particular flame cell group in different specimens, it was found that the number of flame cells was not constant, being greater in larger and presumably older worms. This variation was also observed in different flame cell groups of a single individual. In the largest worms in which all the flame cells and capillary arrangement could be seen with certainty, from ten to twelve were found in a group. In very large specimens, a count of the pigmented capillaries in single groups revealed approximately sixteen. Hopkins (1934b) reported this number for *A. ictaluri* and in a personal communication, has stated that his observation was on the metacercaria. In this stage, the writer has never observed so large a number.

The flame cell pattern shown in Fig. 1 is a composite one representing that observed in young adult worms. Because of the variation in flame cell number, both in corresponding groups of different individuals and different groups in the same individual, it is impossible to express an excretory formula more precisely than an empirical one giving merely the number of flame cell groups.

The Egg and Miracidium (Figs. 2–6)

The egg of *A. ictaluri* is undeveloped when laid and contains several large yolk cells. The shell is operculate and measures from 0.089 to 0.105 by 0.065 to 0.071;

the average length is about 0.10. Development begins after reaching water and a small embryonic mass is discernible after two days at room temperature. By the fifth or sixth day, the eyespot is distinct and after six to eight days the embryo becomes motile. If hatching occurs, it is almost invariably on the morning of the tenth day of incubation at room temperature (about 25° C.). The miracidia are positively phototropic. While swimming or when slightly flattened (Fig. 3), they extend to approximately 0.125 in length. Those killed in hot silver nitrate solution, however, contracted to an average length of 0.065 and a width of 0.040. A distinctive feature of the miracidium is the presence of an elongate, spindle-shaped stylet, the tip of which may be protruded through a minute opening in the center of the terebratorium. The stylet is 0.016 in length.

Cilia are rather uniform in length and distribution. The ciliated epidermal cells of the miracidium (Figs. 4–6) are in four tiers of six, seven, four and two plates respectively from the anterior to posterior end of the larva. Cells of the anterior tier (Fig. 6) are bilaterally symmetrical in arrangement, each half having a dorsolateral, a ventrolateral and a lateral cell. Although the second tier shows no such symmetry, the cells of the third and fourth are so arranged. Each half of the third has a dorsolateral and a ventrolateral epidermal plate while the fourth consists of a pair of lateral cells. The latter are small and, in the strongly contracted silver impregnated specimens, can be seen clearly only in end view (Fig. 5). On each side of these plates, there are two pore-like structures which are plainly visible in these preparations. They are dissimilar in size and lie close together at the junction of the lateral plates of the fourth tier and the ventrolateral and dorsolateral plates of the third. The larger of these probably is the excretory pore, for from it what appears to be the impregnated posterior end of the excretory tubule can be traced anteriorly for a short distance (Fig. 4).

In an *en face* view of silver impregnated specimens (Fig. 6), several features otherwise indistinct are clearly visible. There are six small oval structures, one in a slight indentation in the mid-posterior edge of each cell in the first tier. These can be seen as small, knob-like structures in living specimens and probably are sensory papillae such as those described for other miracidia. In the terebratorium, what appear to be openings of glands are especially distinct (Fig. 6). There are four pairs, all the same size, and sometimes forming an almost complete circle around the central opening for the stylet. More often, however, they are arranged in two bilaterally symmetrical arcuate rows separated dorsally and ventrally by gaps. Two additional pairs, dissimilar in size, are also present in this region. They are situated on each side at the junction of the anterior tips of the lateral and ventrolateral epidermal cells of the first tier and are distinctly separated from the eight more centrally placed ones.

Glandular structures, opening at the anterior end of the miracidium, consist of two masses, one behind the other, and arranged asymmetrically when seen in side view. The shortest of these is dorsally placed and contains four large distinct nuclei. However, the mass appears to be syncytial, for cell boundaries or separate ducts could not be observed. In stained preparations this mass appears as a lighter, almost empty space containing the nuclei. Posterior to this is a second mass with what appears to be a duct extending anteriorly, ventral to that of the first. Like that structure, the second contains four nuclei and cell boundaries were not appar-

ent. The nuclei are smaller, however, than in the first mass and the cytoplasm in stained preparations appears faintly granular. Still further posterior, at and behind the eyespot level, is a third mass which is probably ganglionic in nature, since it occupies the position of such structures described for other miracidia and has no evident connection with the anterior end. Several nuclei are associated with this mass. The arrangement of glandular structures and their openings on the terebratorium are difficult to interpret. The anterior-most glandular mass may correspond to what has been described as the primitive gut in other miracidia, and the posterior may be cephalic glands. Indeed, their arrangement agrees well with that of such structures described by Lynch (1933) for the miracidium of *Heronimus chelydrae,* although he did not interpret the anterior mass as a primitive intestine. However, no cells corresponding to the oxyphilic glands have been observed in the present species. Instead, that region is occupied by a slender sac containing the stylet. The relationship of ducts from these glands to the apparent openings on the terebratorium as described above has not been determined. The openings are visible only in an *en face* view of silver impregnated specimens, but in these, no ducts leading inward from the openings could be seen. The fact that there are eight nuclei in what seem definitely to be glandular structures and eight apparent openings in bilaterally symmetrical rows on the terebratorium suggests that the glands may develop in the miracidium as unicellular structures, each with its own duct and opening and that the identity of the cells is lost by their fusion. However, this would leave unexplained the two additional pairs of what also appear to be openings in the ventrolateral region of the terebratorium. These correspond in position to the pores of the cephalic glands in the miracidium of *Heronimus chelydrae* as described by Lynch. In the present species no evidence of glands leading to these structures has been observed. For that reason, it is suggested that they may be sensory in function. The eyespot of the miracidium is dorsally placed and consists of two fused cups of pigment granules each containing what appears to be a refractile body. The large germinal cells are few in number and begin just posterior to the eyespot. Their cytoplasmic boundaries are distinct and the nuclei contain large, often block-like karyosomes. They are flanked by highly refractile cells which do not appear to be germinal in nature. On each side and at the extreme posterior end of the miracidium is what appears in ventral view of living material to be a single large cell, although each contains two nuclei and may actually be two cells.

The flame cells are laterally placed at or slightly posterior to the middle of the larva. From each, the capillary extends in a tortuous path, first posteriorly and then anteriorly as far as the flame cell where it turns and extends to the excretory pore at the posterior margin of the third tier of epidermal plates.

There is little precise information concerning miracidia of species that are supposedly closely related to *Allocreadium ictaluri.* Because of its early place in the ontogeny of the DIGENEA, it is unfortunate that more attention has not been given to the comparative study of miracidia. This is especially true in regard to the ALLOCREADIOIDEA whose affinities are so much in doubt. For this reason, the present investigation has included a study of the miracidium of *Crepidostomum ictaluri,* a species often encountered in catfish which the writer has examined. The eggs of this species are much smaller than those of *A. ictaluri* and the worm contains only

a few at one time. When handled in the same manner as for the eggs of *A. ictaluri,* they developed normally and hatched on the eighth or ninth day.

In his description, Hopkins (1934b) stated that the miracidium of *C. ictaluri* was ciliated but that the epidermis was not divided into "cuticular" plates. Silver impregnated specimens reveal that this is not the case (Figs. 7–9). The number and arrangement of plates are precisely the same as in the miracidia of *A. ictaluri* except that there are six in the second tier rather than seven. This may be an anomaly, since the author was able to carry through only a few such preparations and variation in epidermal cell numbers has been reported. However, there are certain other differences between the miracidia of *A. ictaluri* and *C. ictaluri* that should be noted, although their significance cannot be evaluated until the structure of other allocreadioid miracidia is better known. In contrast to *A. ictaluri,* the terebratorium of *C. ictaluri* miracidia bears only two structures presumed to be glandular openings and apparently lacks the six sensory papillae between the first and second tiers of epidermal cells. Furthermore, a stylet is absent and the structures interpreted as the excretory pores, instead of being accompanied by one smaller structure is flanked by a pair of them (Cf. Figs. 7 and 4, 8 and 5, 9 and 6). Silver impregnated specimens of *C. ictaluri* miracidia measure 0.035 long and 0.023 wide, dimensions which are much less than those given by Hopkins. However, this difference may be explained by the contraction which occurs when living miracidia are placed in hot silver nitrate solution.

The Redia (Fig. 10)

Since only rediae containing cercariae have been observed and there have been only a few as yet unsuccessful attempts to infect snails with miracidia, it is not known whether there may be more than one redial generation. In almost every infected snail that has been examined, all of the rediae have been in an advanced stage of development, containing cercariae and cercarial embryos. Very young rediae have been found in but one mollusc and from that only two were recovered from the digestive gland, along with older rediae. No indication of a sporocyst has been observed.

The rediae of *A. ictaluri* are found in large numbers in the liver of the snail, although a few occur also in the mantle and gills. When first removed from the snail, they may be somewhat motile, but their movements are due largely to their contained brood of active cercariae. Rediae measure up to 1.5 in length. The pharynx averages about 0.05 in diameter and the simple intestine is one-third to one-half the length of the body. As many as twenty cercariae, sufficiently developed to possess eyespots, have been observed in a single redia, though in most instances, there are less than ten such larvae in addition to embryos in all stages of development.

No evidence of a birth pore has been observed in rediae nor has the escape of cercariae from them been seen, although many rediae have been observed for extended periods in an effort to determine how this may take place.

The Cercaria (Figs. 11–16)

The cercaria of *A. ictaluri* is an ophthalmocercaria developing in rediae in the liver of *Pleurocera acuta.* In the laboratory, emergence from the snail occurs in the early morning. For a short time, cercariae respond positively to light, but after ap-

proximately an hour become negatively phototropic. The larva is propelled by smooth figure-eight lashing of the tail which precedes the body. However, the body is flexed ventrad so that its anterior end tends to point in the direction in which the larva is swimming.

Fully contracted, the body is 0.14 long and 0.12 wide; when not swimming, it may extend to 0.25 in length and 0.09 in width. Prominent cuticular spines occur in transverse rows at the anterior end of the body and small papillae, each bearing a minute seta, are scattered over the body surface. Although not evenly distributed, these papillae are remarkably uniform in number and location as revealed by silver impregnation (Figs. 12–15). They are especially numerous in the vicinity of the suckers and several are distributed in a bilaterally symmetrical pattern over the cuticle on each side from the ventro-lateral to the dorso-lateral regions while the mid-dorsal and mid-ventral regions (except near the suckers) are devoid of them. Some of the structures appearing on the anterior lip of the oral sucker in silver impregnated specimens (Figs. 12 and 13) may be openings of the cephalic glands. The tail varies in length from 0.10 to 0.30, depending on the degree of contraction. Minute folds appear as transverse annulations along the edge. The cuticle of the tail bears fine setae set in papillae similar to those on the body. As in that location, they are remarkably constant in number and bilaterally symmetrical in distribution.

In living material, the oral sucker is subterminal, measuring 0.048 (over 0.05 under light cover glass pressure) in diameter. The ventral sucker is slightly posterior to the middle of the body and measures 0.054 (0.06 under light cover glass pressure) in diameter. It forms a decided protrusion of the ventral surface as seen in side view.

A short prepharynx is present and the prominent pharynx measures 0.022 in diameter. The digestive tract was not observed much beyond the bifurcation of the intestine in living and stained cercariae. However, in specimens impregnated with silver, ceca extending almost to the posterior end of the body were clearly seen.

The eyespots are located dorsally and, in an extended or slightly flattened specimen, at the level of the anterior edge of the pharynx. Between the pharynx and acetabulum the body is almost filled with glands whose ducts extend anteriorly in four bundles of three ducts each, close to the dorsal surface of the body. On each side, the median-most bundle passes between the eyespot and the pharynx while the outer group is lateral to the eyespot. Attempts to determine the number of cephalic gland cells were unsuccessful; their nuclei were indistinct and the cells were so lobulated that they could not be counted with certainty. However, there probably is the same number of gland cells as ducts, viz., six on each side; that each cephalic gland cell has its own duct has been reported for many species of cercariae and this probably is true of some for which fewer ducts than gland cells have been described.

In stained specimens, the genital primordium is a distinct mass, lying dorsal to the left side of the ventral sucker and immediately anterior to the excretory vesicle. The excretory vesicle (Figs. 11 and 16) is sac-like and may extend to the mid-acetabular level. Its anterior end is deflected to the right by the genital primordium when the body is extended or slightly flattened. The wall is only moderately thick with rather even internal and external contours and seems to be composed of small, block-like, granular cells, although nuclei of such were not observed. The bladder is always filled with a variable number of refractile concretions measuring 0.01 to

0.18 in diameter. They appear to have been formed by successive deposition of concentric layers of transparent material around one to four central masses. As many as thirty such concretions may be present. They are soluble in acids.

The two main collecting canals are ciliated and empty into the vesicle separately. From that point, each follows a tortuous path to the level of the anterior edge of the acetabulum or slightly beyond. There it turns abruptly and proceeds posteriorly as far as the mid-acetabular level where it receives the anterior and posterior collecting tubules. In spontaneously emerging cercariae, the capillaries and flame cells are, for the most part, so obscured by glands and refractile droplets of the body that only an occasional flame cell can be seen. It was found, however, that in not quite mature cercariae obtained by cracking the snail, the flame cells and many of the capillaries could be observed.

As in the adult, there are six groups of flame cells of which the three anterior empty into the anterior collecting duct, the remainder in the posterior. Certain flame cells were seen consistently, because they were always beating and were slightly larger than others. This was especially true of the anterior- and posterior-most flame cells of the body. Others which frequently were inactive and somewhat smaller could be seen only in an occasional specimen. Since larvae obtained by cracking snails were used for these observations, the fact that the number of flame cells in certain groups apparently was not constant may have been due to differences in the degree of development of the cercariae. Thus, usually three, but sometimes four flame cells were seen in the first group and six to nine in the second, whereas there usually were observed five, four, five, and six in the third to sixth groups consecutively. Although capillaries could be traced for a distance from most of the flame cells, their connections were clear only in the case of the two anterior- and the two posterior-most flame cells of the system on each side.

The Metacercaria (Figs. 17 and 18)

All of the unionid bivalves examined from the Tippecanoe River at Battleground, Indiana, and most of those from the Wabash River at Lafayette have been found to be infected with metacercariae of *A. ictaluri*. The cysts occur almost exclusively in the inner tissues of the thick mantle edge and are restricted to the anterior region rather than near the siphons. Between 500 and 600 cysts were estimated to occur in one naturally infected bivalve that was examined and much heavier infections were observed in large mussels.

The cyst increases in size from approximately 0.13 in diameter at the time of encystment to almost 0.4; the diameter of the largest cysts found in a natural infection was 0.39. The cyst wall is at first colorless, but becomes orange-brown in the oldest metacercariae. Most of the cysts, however, are light orange and measure about 0.3 in diameter. They are enclosed in a dense white tissue capsule of host origin. This outer covering is slightly irregular in outline and averages about 0.5 in diameter. It does not separate cleanly from the tissue of the mantle.

The cyst is not especially tough or difficult to remove. When released from it, the largest metacercaria observed was 0.7 in length when relaxed and not under pressure. The majority of such worms, however, measure 0.3 in length by 0.2 in width when free and lying inactive in saline; many are even smaller.

The excretory vesicle of the metacercaria is greatly distended with refractile

bodies forming a dark mass that is easily detected in the intact cyst. This mass is expelled soon after excystment and is not seen in worms taken from fish. The eyespot pigment becomes more and more dispersed as the metacercariae grow. In the largest ones the alimentary tract is completely developed and the gonads and the cirrus sac are distinguishable. Of worms recovered from catfish two and one half weeks after being fed naturally infected molluscs, the largest were sexually mature with active sperms, although eggs were not yet present in the uterus. Many smaller, immature individuals were also present. This variation corresponds to that of the metacercariae at the time they were ingested.

DISCUSSION

Nicoll first used the superfamily name, ALLOCREADIOIDEA, in compiling the section on Vermes in the Zoological Record for 1934. In this group, he included as families the ALLOCREADIIDAE Stossich, 1904, OPECOELIDAE Ozaki, 1925, COITOCAECIDAE Ozaki, 1929, SPHAEROSTOMATIDAE Thapar and Dajal, 1934, MEGAPERIDAE Manter, 1934 (syn. EURYPERIDAE Manter, 1933), LEPOCREADIIDAE Nicoll, 1934, ACANTHOSTOMATIDAE Nicoll, 1934, and ACANTHOCOLPIDAE Lühe, 1909. Since 1934, four additional families have been erected for trematodes presumably belonging to the ALLOCREADIOIDEA or resembling members of that group, but which would not fit into pre-existing families. These are the SPHINCTEROSOMATIDAE Yamaguti, 1937, WARETREMATIDAE Srivastava, 1937, NOTOPORIDAE Yamaguti, 1938, and MEGASOLENIDAE Skrjabin, 1942. Of these, Manter (1947) has recently merged the families SPHINCTEROSOMATIDAE and NOTOPORIDAE with the OPECOELIDAE. Although this reduces the number of families to be considered in a discussion of the ALLOCREADIOIDEA, the group still remains in a state of extreme confusion.

It has been pointed out by Manter (1940) that there exist similarities between members of the ALLOCREADIOIDEA and certain bizarre worms which superficially resemble the amphistomes and for that reason have been assigned to the superfamily PARAMPHISTOMOIDEA Stiles and Goldberger, 1910, by Travassos (1934) and Ozaki (1937). Cable and Hunninen (1942) studied further the descriptions of these amphistome-like forms and concluded that their peculiar adult structure was quite possibly due to disproportionate growth of fore- and hind-body during post-cercarial development. They pointed out that, by considering the presence of lymph channels a morphological specialization that does not necessarily imply close relationship, one might include under the ALLOCREADIOIDEA the families GYLIAUCHENIDAE Ozaki, 1933, and OPISTHOLEBETIDAE Fukui, 1929. Cable and Hunninen also suggested that possibly another family, the CEPHALOPORIDAE Travassos, 1934, hitherto assigned to the PARAMPHISTOMOIDEA, might eventually be transferred to the ALLOCREADIOIDEA. Manter (1940) allocated the GYLIAUCHENIDAE to the superfamily ALLOCREADIOIDEA, but expressed the view that the family has amphistome affinities. Final disposition of these peculiar groups must await further investigations, especially life history studies, none of which have been reported to date.

The family ACANTHOCOLPIDAE appears to be a better defined group although there is disagreement concerning the genera that should be assigned to it and concerning its relationship to the ALLOCREADIOIDEA. Of the genera allocated to the ACANTHOCOLPIDAE by Cable and Hunninen (1942), the life cycle of a species of only one, *Stephanostomum tenue* Linton, has been demonstrated experimentally,

although the metacercariae of others are known to occur in fishes. Martin (1939) found that an ophthalmoxiphidiocercaria, developing in rediae in the liver of the marine snail, *Nassa obsoleta,* was ingested by fish. The larva migrated from the intestine and encysted in the mesenteries and liver. He fed the encysted stage to fish and recovered immature *S. tenue* from the intestine.

Cable and Hunninen (1942) traced the life cycle of *Deropristis inflata* and on the basis of their observations of both larval and adult morphology, excluded the genus *Deropristis* from the ACANTHOCOLPIDAE, transferring it to the LEPOCREADIIDAE. This species is discussed further in connection with that group. Dawes (1946), however, has preferred to retain *Deropristis* in the ACANTHOCOLPIDAE where it has reposed for many years.

The family ACANTHOSTOMATIDAE Poche, 1925, was included by Nicoll in the ALLOCREADIOIDEA, presumably for convenience, since three of the genera, which Poche assigned to the family, have been transferred unequivocally to the HETEROPHYIDAE by Mueller and Van Cleave; these genera were *Cryptogonimus* Osborn, 1903, *Caecincola* Marshall and Gilbert, 1905, and *Allocanthocasmus* Van Cleave, 1922. The life cycle of *Caecincola parvulus,* which has subsequently been traced, fully justifies this disposition. The remaining genera placed in the ACANTHOSTOMATIDAE by Poche and those subsequently assigned to it constitute a group which is morphologically diverse and probably does not represent a natural group of trematodes as closely interrelated as those assigned to the same family should be.

The OPECOELIDAE and COITOCAECIDAE may be discussed briefly together, since there is now abundant evidence that they are not distinct. For that reason, the two will hereinafter be referred to as the OPECOELIDAE. Although certain of the adults of species assigned to this group are remarkably similar to the genus *Allocreadium,* type of the family ALLOCREADIIDAE, life history studies reveal that they are removed from the ALLOCREADIIDAE to the extent of constituting at least a distinct family. The cercaria is a microcercous form which Dollfus (1913) pointed out should be regarded as a distinct larval type and not a stumpy-tailed modification of other kinds of cercariae. Several life histories of larvae of this type have been traced and indicate that, with the possible exception of a few little known and perhaps aberrant genera, those at present allocated to the OPECOELIDAE constitute a natural group of related trematodes. A comprehensive review of the family has been given recently by Manter (1947) who has recognized four subfamilies.

The LEPOCREADIIDAE as a family group was first utilized without being defined by Nicoll in the Zoological Record for 1934, and has subsequently been employed by various investigators. In their studies on the life cycle of *Deropristis inflata,* Cable and Hunninen (1942) attempted to define the LEPOCREADIIDAE as a natural group and included in it forms having trichocercous cercariae developing in gastropods, encysting in invertebrates, maturing in fishes, and possessing many flame cells. They proposed the subfamily DEROPRISTINAE to contain the genus *Deropristis* which they excluded from the ACANTHOCOLPIDAE. As mentioned above, Dawes (1946) preferred to take a more conservative viewpoint, leaving *Deropristis* in the ACANTHOCOLPIDAE and considering the LEPOCREADIINAE Odhner, 1905, as a subfamily of the ALLOCREADIIDAE.

The life histories of few other LEPOCREADIIDAE have been demonstrated. *Lepocreadium album* Stossich was shown by Palombi (1937) to develop in marine snails,

Nassa spp., and the cercaria, a trichocercous type, encysted in nudibranchs. The adult parasitized fishes. The next year, Martin (1938) traced the life history of *L. setiferoides* (Miller and Northup, 1926) and found that the cycle of this species was very similar to that of *L. album,* having a trichocercous cercaria developing in rediae in the snail, *Nassa obsoleta.* Encystment occurred in a turbellarian, *Procerοides warreni,* and an annelid, *Spio.* Young adults were obtained experimentally in the intestine of the flounder. The cercaria of *Opechona bacillaris* has also been reported to be a trichocercous type (Lebour, 1916).

Hopkins (1937) described two new ophthalmocercariae of what he termed an anallocreadiine type, distinguished by absence of a stylet and possession of a slender tail with three pairs of lateral papillae, each bearing a single seta. These larvae developed in rediae in the snail, *Amnicola peracuta,* and encysted in the bivalve, *Musculium ferrissi,* where they developed to stages recognizable as the young of *Microcreadium parvum* Simer, 1929 and *Anallocreadium armatum* MacCallum, 1895. The striking similarity between the cercariae described by Hopkins and the larva of *Deropristis inflata* was in part the basis on which Cable and Hunninen (1942) transferred *Deropristis* from the Acanthocolpidae to the Lepocreadiidae.

Although Nicoll did not assign the family Monorchiidae Odhner, 1911, to the superfamily Allocreadioidea, members of this family have several features in common with groups discussed above and which were so allocated by Nicoll. Indeed, a relationship between the Monorchiidae and Allocreadiidae was suggested by Srivastava (1939) in establishing the family Waretrematidae for his genus *Waretrema* which he described as being near the Monorchiidae and the Allocreadiidae. He also mentioned the family Haploporidae as being related to the Waretrematidae.

Only one life history has been traced in the Monorchiidae. Martin (1938) described an ophthalmocercaria developing in sporocysts in the bivalves *Cumingia tellinoides* and *Tellina tenera.* This larva possessed a simple, sac-shaped bladder and a tail with peculiar lateral lappets. After a brief swimming period, it encysted in the same bivalves. He named the larva *C. cumingiae* and later (1940) determined in feeding experiments that its adult stage was an undescribed species, *Monorcheides cumingiae.*

In discussing the family Allocreadiidae *sensu stricto,* one is immediately confronted with many problems which cannot be resolved with information available at the present time. In the first place, there has been no generally accepted definition of the family, some authors including in it as subfamilies one or more of the groups discussed above as families. Perhaps the greatest single difficulty is that many genera that have been proposed have been so poorly characterized as to make their assignment to supra-generic categories almost a matter of pure conjecture, even if there were general agreement as to the rank of those categories.

Taxonomic schemes and especially generic diagnoses have been based almost entirely on adult structure and it has become increasingly apparent from life history studies that there has been convergence of distantly related species as well as divergence of closely related ones, making the validity of taxonomic categories based solely on adult morphology a matter of considerable doubt. On the other hand, certain investigators have placed much emphasis on larval types, especially the cercaria. However, this stage has no counterpart in the life histories of turbellarians, presum-

ably descended along with the trematodes from a common ancestral group. This being the case, the cercaria with its modifications for larval life and penetrating new hosts may, as a criterion of affinity, be as subject to criticism as is the adult stage. Yet it is a fact that in such groups as the OPISTHORCHIOIDEA, PLAGIORCHIOIDEA, STRIGEOIDEA, and others, the cercarial type is remarkably uniform, although there exist in almost every case, examples of aberrant and modified larvae as well as adults. Two sources of evidence as to taxonomic affinities have been generally neglected, viz., host-parasite relationship and the detailed structure of the miracidium, the earliest ontogenetic stage.

By excluding those genera that have been or properly may be assigned to families already discussed, there remains in the restricted family Allocreadiidae the following genera: *Allocreadium* Looss, 1900; *Choanostoma* Yamaguti, 1934; *Crepidostomum* Braun, 1900; *Creptotrema* Travassos, Artigas and Pereira, 1928; *Diplobulbus* Yamaguti, 1934; *Eucreadium* Dayal, 1944; *Gnathomyzon* Crowcroft, 1945; *Indocreadium* Srivastava, 1943; *Kaurma* Chatterji, 1936; *Laureriella* Skrjabin, 1922; *Neopodocotyle* Dayal, 1944; *Parvacreadium* Manter, 1940; *Phyllotrema* Yamaguti, 1934; *Pycnadenoides* Yamaguti, 1938; and *Trematichtys* Vas, 1932. A critical study of some of the above genera would almost certainly reveal that they, too, properly belong in other families.

Until the present study, the life cycle of a few species of only one of the above genera, i.e., *Crepidostomum*, has been demonstrated experimentally. These include *C. cooperi* by Hopkins (1934b), *C. cornutum* by Ameel (1937) and *C. farionis*, traced partially in Europe by Brown (1927) and more completely in the United States by Crawford (1943). Portions of life cycles of these and other species of *Crepidostomum* have been observed by various other investigators. In all of these life cycles, there is a remarkable uniformity of the larval types and of host-parasite relationship of the various stages. Their larvae are ophthalmoxiphidiocercariae developing in rediae in the liver of sphaeriid bivalves. Furthermore, all of them encyst in arthropods and develop to maturity in the intestine of fresh water fishes.

Although the present study has a bearing on the entire family Allocreadiidae, it concerns especially the type genus, *Allocreadium*. The descriptions of some species allocated to this genus are inadequate; for this reason, their taxonomic status is uncertain. However, the writer is not in a position to re-evaluate the group for reasons that will become apparent in discussing the status of *A. ictaluri* as a valid member of the genus.

On the basis of adult structure alone, with the possible exception of the excretory system, *A. ictaluri* would certainly seem to be congeneric with *A. isoporum*, type species of the genus *Allocreadium*. However, when stages in the life cycle of *A. ictaluri* and host-parasite relationship of its larvae are considered, it may indeed be doubted whether the species belongs in the genus *Allocreadium*. From what is known of the life histories of other trematodes, e.g., the species of *Crepidostomum*, it would be expected that two members of the same genus would have cercariae that are more similar than are the ones here demonstrated to be the larva of *A. ictaluri* and that supposed to be the cercaria of *A. isoporum*. Furthermore, it seems especially unlikely that species in the same genus would have such different molluscan hosts as sphaeriid bivalves and prosobranch gastropods. Such a host-parasite relationship would be a decided innovation among the DIGENEA. However, to al-

locate *A. ictaluri* to another genus seems inadvisable at present, not so much because of doubt concerning the validity of larval characters or host-parasite relationship as the fact that the life history of *A. isoporum* must be considered unproved. Neither Looss (1894) nor Dollfus (1949) demonstrated the life cycle experimentally and the postulation of larval and adult affinities on the basis of morphological comparisons is far from proof. The author has seen another trematode, as yet undescribed, which, on the basis of comparative morphology, could perfectly well be postulated to be the adult of the cercaria of *A. ictaluri.* Yet this worm, occurring in sturgeons from the Wabash River, is not even in the family ALLOCREADIIDAE.

In respect to morphology and host-parasite relationship, the cercaria supposed to be that of *A. isoporum* is much more like the larva of *Crepidostomum* than that of *A. ictaluri* which, on the other hand, has many features in common with the cercaria of *Deropristis inflata,* the "Anallocreadiine" larvae of Hopkins (1937) and even the distinctly trichocercous forms. Among these points of similarity are development in rediae in prosobranch gastropods, absence of a stylet, and structure of the excretory vesicle and the excretory pattern. The almost constant number and arrangement of the caudal papillae and bristles, delicate though they are in the cercaria of *A. ictaluri,* are suggestive of affinities with the trichocercous forms. The author has prepared silver impregnated specimens of ophthalmoxiphidiocercariae from sphaeriids and found that the tail is entirely devoid of such papillae. This observation increases the probability of such structures on the tail of *A. ictaluri* cercariae being indicative of trichocercous affinities.

Observations reported above show that the miracidia of *A. ictaluri* and *Crepidostomum ictaluri,* though similar, show differences in the number of epidermal cells in the second tier, in what appear to be openings of glands in the terebratorium, and in structures associated with the excretory pores. To evaluate the significance of these differences would require knowledge of the miracidia of many other genera, including representatives of the LEPOCREADIIDAE and ACANTHOCOLPIDAE. Such information unfortunately is not at hand and should be one of the main objectives of further studies on life histories of the still highly controversial allocreadioid trematodes.

SUMMARY

The life history of *A. ictaluri* has been demonstrated by means of controlled experiments. The cercaria is biocellate and lacks a stylet; both body and tail bear inconspicuous papillae, symmetrically arranged and provided with delicate setae. The cercariae develop in simple rediae in the digestive gland of *Pleurocera acuta* (Say), encyst in bivalves and develop to maturity in the intestine of various species of catfish. The miracidia of *A. ictaluri* and *Crepidostomum ictaluri* are described and compared. The taxonomy of *A. ictaluri* and the ALLOCREADIIDAE in general is discussed.

REFERENCES

AMEEL, D. J. 1937 The life history of *Crepidostomum cornutum* (Osborn). J. Parasit. **23:** 218–220.

BROWN, F. J. 1927 On *Crepidostomum farionis* O. F. Müller (=*Stephanophiala laureata* Zeder), a distome parasite of the trout and grayling. I. The life history. Parasitol. **19:** 86–99.

CABLE, R. M. AND HUNNINEN, A. V. 1942 Studies on *Deropristis inflata* Molin, its life history and affinities to trematodes of the family Acanthocolpidae. Biol. Bull. **82:** 292–312.

CLARK, H. W. AND WILSON, C. B. 1911 Mussel fauna of the Maumee River. U. S. Bureau Fish. Doc. 757: 1–72.

CRAWFORD, W. W. 1943 Colorado trematode studies. 1. A further contribution to the life history of *Crepidostomum farionis* (Müller). J. Parasit. **29:** 379–384.

DAWES, B. 1946 The trematoda—with special reference to British and other European forms. Cambridge. 644 pp.

DOBROVOLNY, C. G. 1939 The life history of *Plagioporus lepomis*, a new trematode from fishes. J. Parasit. **25:** 461–469.

DOLLFUS, R. P. 1913 *Cercaria pachycera* Diesing et les cercaires a queue dité en moignon IX Congres Internat. Zool. Monaco (Résumés des communications): 38–42.

————1949 Sur une cercaire ophthalmoxiphidiocerque *Cercaria isopori* A. Looss 1894 et sur la délimitation des Allocreadioidea. Ann. Parasit. **24:** 424–435.

HOPKINS, S. H. 1934a The parasite inducing pearl formation in American fresh-water unionidae. Science **79:** 385–386.

————1934b The papillose Allocreadiidae. Ill. Biol. Monogr. **13:** 1–80.

————1937 A new type of Allocreadiid cercaria: the cercaria of *Anallocreadium* and *Microcreadium*. J. Parasit. **23:** 94–97.

————1941 The excretory systems of *Helicometra* and *Cymbephallus* (Trematoda), with remarks on their relationships. Trans. Amer. Micr. Soc. **60:** 41–44.

LEBOUR, M. V. 1916 Medusae as hosts for larval trematodes. J. Marine Biol. Assn. United Kingdom **11:** 57–59.

LOOSS, A. 1894 Die Distomen unserer Fische and Frösche. Neue Untersuchungen über Bau und Entwickelung des Distomenkörpers. Biblioth. Zool. **16:** 1–296.

————1899 Weitere Beiträge zur Kenntniss der Trematoden-Fauna Ägyptens. Zool. Jahrb., System. **12:** 521–784.

————1900 Nachträgliche Bemerkungen zu den Namen der von mir vorgeschlagenen Distomengattungen. Zool. Anz. **23:** 601–608.

————1901 Ueber einige Distomen der Labriden des Triester Hafens. Centralbl. Bakt. I Abt. **29:** 398–405.

————1902 Ueber neue und bekannte Trematoden aus Seeschildkröten. Zool. Jahrb., System. **16:** 411–889.

LYNCH, J. E. 1933 The miracidium of *Heronimus chelydrae* MacCallum. Quart. J. Micro. Sci. **76:** 13–36.

MANTER, H. W. 1940 Digenetic trematodes of fishes from the Galapagos Islands and the neighboring Pacific. Allan Hancock Pacific Expedition **2:** 329–497.

————1947 The digenetic trematodes of marine fishes of Tortugas, Florida. Amer. Midl. Natur. **38:** 257–416.

MARTIN, W. E. 1938 Studies on the trematodes of Woods Hole. The life cycle of *Lepocreadium setiferoides* (Miller and Northup), Allocreadiidae, and the description of *Cercaria cumingiae* n. sp. Biol. Bull. **75:** 463–474.

————1939 Studies on the trematodes of Woods Hole. II. The life cycle of *Stephanostomum tenue* (Linton). Biol. Bull. **77:** 65–73.

————1940 Studies on the trematodes of Woods Hole. III. The life cycle of *Monorcheides cumingiae* (Martin) with special reference to its effect on the invertebrate host. Biol. Bull. **79:** 131–144.

MATHIAS, P. 1937 Cycle évolutif d'un trématode de la famille des Allocreadiidae Stossich [*Allocreadium angusticolle* (Hausmann)]. C. R. Acad. Sci. **205:** 626–628.

MUELLER, J. F. AND VAN CLEAVE, H. J. 1932 Parasites of Oneida Lake fishes. II. Descriptions of new species and some general taxonomic considerations, especially concerning the trematode family Heterophyidae. Roosevelt Wild Life Ann. **3:** 78–137.

NICOLL, W. 1909a Studies on the structure and classification of the digenetic trematodes. Quart. J. Micr. Sci. **53:** 391–487.

————1909b A contribution towards a knowledge of the entozoa of British marine fishes. Ann. Mag. Nat. Hist. Ser. 8, **4:** 1–25.

————1910 On the entozoa of fishes from the Firth of Clyde. Parasitol. **3:** 322–359.

————1915 A list of the trematode parasites of British marine fishes. Parasitol. **7:** 339–378.

ODHNER, T. 1901 Revision einiger Arten der Distomengattung *Allocreadium* Looss. Zool. Jahrb., System. **14:** 483–520.

————1902 Mitteilungen zur Kenntnis der Distomen. II. Centralb. Bakt. I Abt. **31:** 152–162.

————1906 Die Trematoden des arktischen Gebietes. Fauna Arctica **4:** 291–372.

OZAKI, Y. 1937 Studies on the trematode families Gyliauchenidae and Opistholebetidae, with special reference to lymph system. II. J. Sci. Hiroshima Univ. Zool. **5:** 200–212.

PALOMBI, A. 1937 Il ciclo biologico di *Lepocreadium album* Stossich sperimentalmente realizzato. Riv. Parassit. **1**: 1–12.

PEARSE, A. S. 1924 Observations on parasitic worms from Wisconsin fishes. Trans. Wis. Acad. Sci., Arts and Letters **21**: 147–160.

SRIVASTAVA, H. D. 1939 The morphology and systematic relationships of a new parasite, *Waretrema pisicola*, gen. and sp. nov., referable to a new family Waretrematidae, of digenetic trematodes. Ind. J. Vet. Sci. Delhi. **9**: 169–172.

STOSSICH, M. 1900 Osservazioni elmintologiche. Boll. Soc. Adriat. Sci. Nat. Trieste **20**: 89–103.

———1904 Alcuni distomi della collezione elmintologica del museo zoologico di Napoli. Ann. Mus. Zool. R. Univ. Napoli (Nuova Serie) **23**: 1–14.

———1905 Note distomologiche. Boll. Soc. Adriat. Sci. Nat. Trieste **22**: 211–227.

TRAVASSOS, L. 1934 Synopse dos Paramphistomoidea. Mem. Inst. Oswaldo Cruz **29**: 19–178.

VAN CLEAVE, H. J. AND MUELLER, J. F. 1934 Parasites of Oneida Lake fishes. III. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Ann. **3**: 159–334.

WALLIN, I. E. 1909 A new species of the trematode genus *Allocreadium* with a revision of the genus and a key to the subfamily Allocreadiinae. Trans. Amer. Micr. Soc. **29**: 50–64.

WILSON, C. B. AND CLARK, H. W. 1911 Mussel fauna of the Kankakee Basin. U. S. Bur. Fish. Doc. **758**: 1–52.

YAMAGUTI, S. 1938 Studies on the helminth fauna of Japan. Part 21. Trematodes of fishes, IV. Kyoto: 139 pp.

PLATE I

FIG. 1. *Allocreadium ictaluri,* adult; excretory system on left side, detail of other structures on right side and center; ventral view.

FIG. 2. *A. ictaluri,* egg.

FIG. 3. *A. ictaluri,* miracidium, internal anatomy; slightly flattened by cover glass pressure; ventral view.

FIG. 4. *A. ictaluri,* miracidium; treated with silver nitrate to show epidermal plates; lateral view.

FIG. 5. *A. ictaluri,* miracidium; epidermal plates; posterior view.

FIG. 6. *A. ictaluri,* miracidium; epidermal plates; anterior view.

FIG. 7. *Crepidostomum ictaluri,* miracidium; epidermal plates; dorsal view.

FIG. 8. *C. ictaluri,* miracidium; epidermal plates; posterior view.

FIG. 9. *C. ictaluri,* miracidium; epidermal plates; anterior view.

PLATE II

All figures concern *Allocreadium ictaluri.*

FIG. 10. Redia.

FIG. 11. Cercaria, ventral view.

FIG. 12. Cercaria; treated with silver nitrate to delineate position of setae on cuticle; lateral view.

FIG. 13. Cercaria; setae, ventral view.

FIG. 14. Cercaria; setae, dorsal view.

FIG. 15. Tail of Cercaria; to show variation from the basic, symmetrical position of setae on the tail as shown in Fig. 14; dorsal view.

FIG. 16. Cercaria; outline to show excretory system; ventral view.

FIG. 17. Metacercaria, three days after encystment.

FIG. 18. Metacercaria; old specimen from mantle of naturally infected mussel.

PLATE I

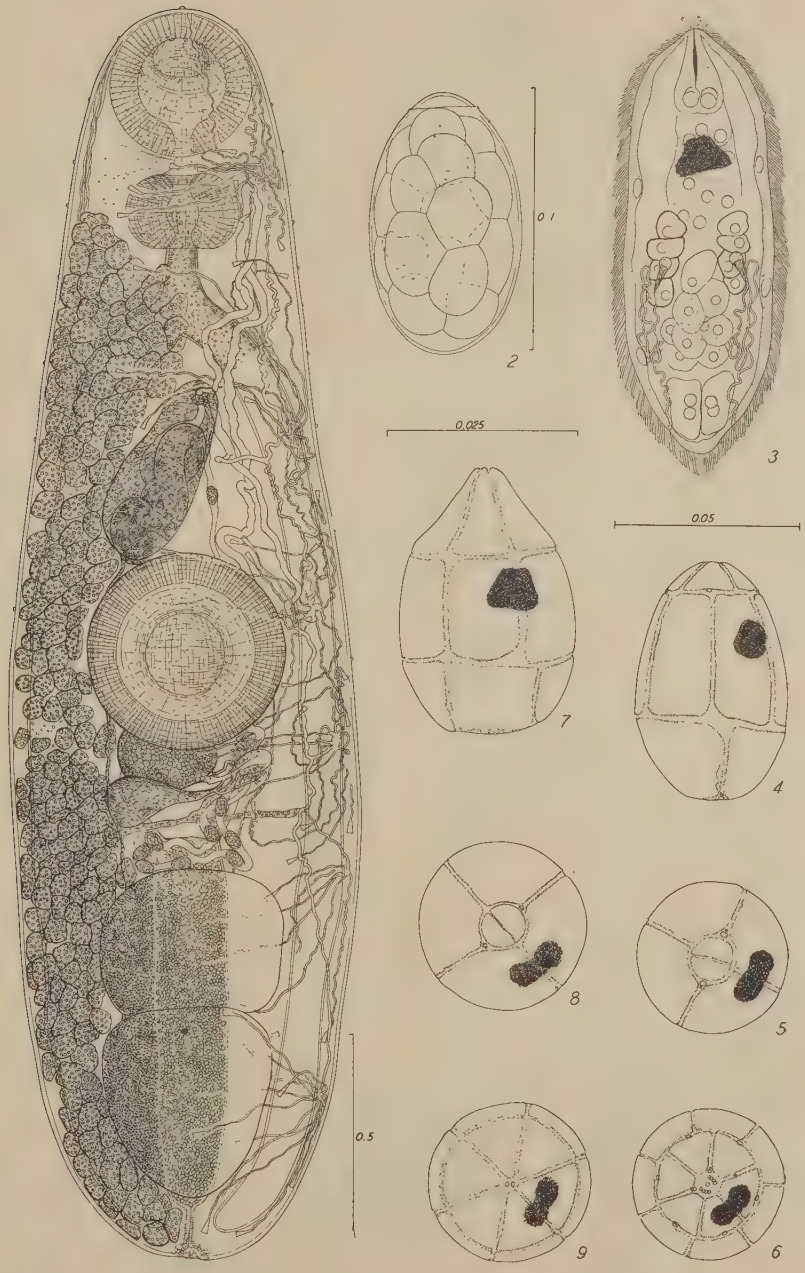


PLATE II

TWO NEW HEMOFLAGELLATES (GENUS *CRYPTOBIA*) FROM SOME WESTERN WASHINGTON TELEOSTS

MAX KATZ[1]

Biologist, Environmental Health Center, U. S. Public Health Service, Cincinnati 2, Ohio

Some hemoflagellates were found in Giemsa stained blood films prepared during a hematological study of the silver salmon, *Oncorhynchus kisutch* (Walbaum). Subsequent examination showed these organisms belonged to the genus *Cryptobia* Leidy 1846 (= *Trypanoplasma* Laveran and Mesnil, 1901). These *Cryptobia* were first found in the blood of adult silver salmon that were being spawned in the Washington State Department of Fisheries hatchery at Soos Creek, near Auburn, King County, Washington. These organisms were later found in spawning silver salmon taken at Burns Creek, another tributary of the Green River and in fingerling silver salmon taken in Swamp Creek near Seattle, King County, Washington.

Cryptobias were found in the adult salmon during the months of November through February. A search during the summer of 1948 for the same or related hemoflagellates in other Soos Creek fish was rewarded by the discovery of another species of *Cryptobia* in *Cottus rhotheus* (Rosa Smith) and *Cottus aleuticus* Gilbert. Cottids of the same species taken in Swamp Creek also had these flagellates. *Cryptobia,* though present as blood and intestinal parasites of several fresh-water and marine fish, have not been reported from any Pacific basin salmonid or cottid. Because of their presence in these new hosts and some demonstrable morphometric differences, these *Cryptobia* were judged to be two new species and are herein recorded.

ACKNOWLEDGMENTS

The author wishes to recognize the kind assistance of Dr. J. E. Lynch, Professor of Fisheries, at the University of Washington, who was the first to recognize that the unusual "leucocytes" in the stained blood film were blood parasites belonging to undescribed species. Dr. Lynch's encouragement and guidance are gratefully acknowledged.

TECHNIQUE

Although cryptobias were first observed in air-dried films that had been stained with Giemsa's, most of the organisms studied were fixed with Schaudinn's and stained with iron-alum hematoxylin. Occasionally, a very satisfactory preparation was obtained with Giemsa's but most of the slides prepared by this method were unsatisfactory because of the distortion of the preserved organism's blepharoplast and nucleus.

Observations of the living cryptobiae were easily made. Often the living parasite could be found in a drop of fish blood dispersed in saline. Because of the greatly decreased clotting ability of the blood of the spawning salmon, a great amount of blood could be prevented from clotting by the use of relatively small volumes of 0.5 per cent potassium oxalate solution and could be preserved for ade-

Received for publication, May 27, 1950.

[1] This study was supported by a grant from the U. S. Public Health Service, Division of Research Grants and Fellowships and was made at the School of Fisheries, University of Washington, Seattle.

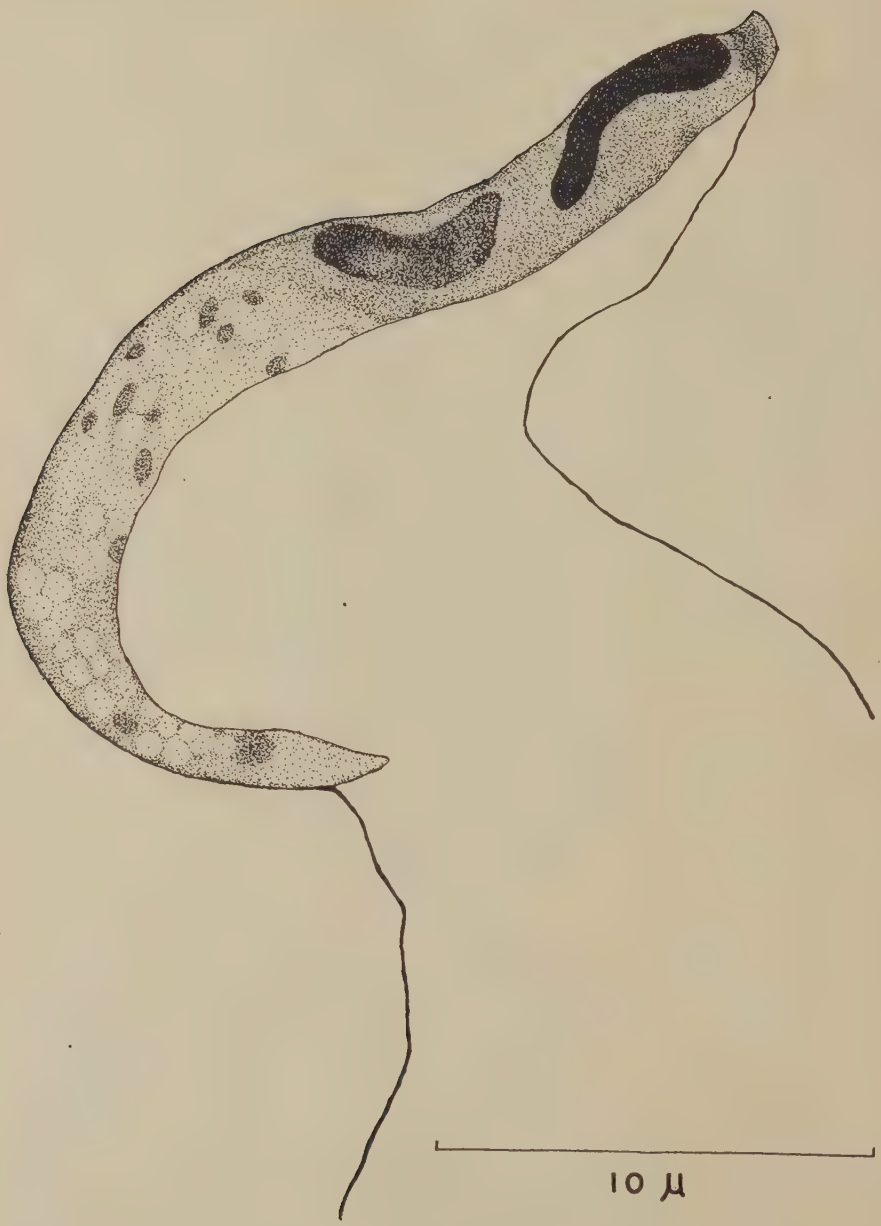


FIG. 1. *Cryptobia salmositica* n. sp. From photomicrograph of organism stained with Giemsa's.

quate study of the living organism. *Cryptobia* would survive for at least ten days if the blood suspension was kept under ordinary refrigeration.

Genus: CRYPTOBIA Leidy

Cryptobia salmositica new species

Figure 1

Specific diagnosis: (Average body dimensions based on the measurement of 196 specimens from 10 salmon.) Body length, 14.94 (range 6.0–25.0) microns, standard deviation (s.d.) 3.34;

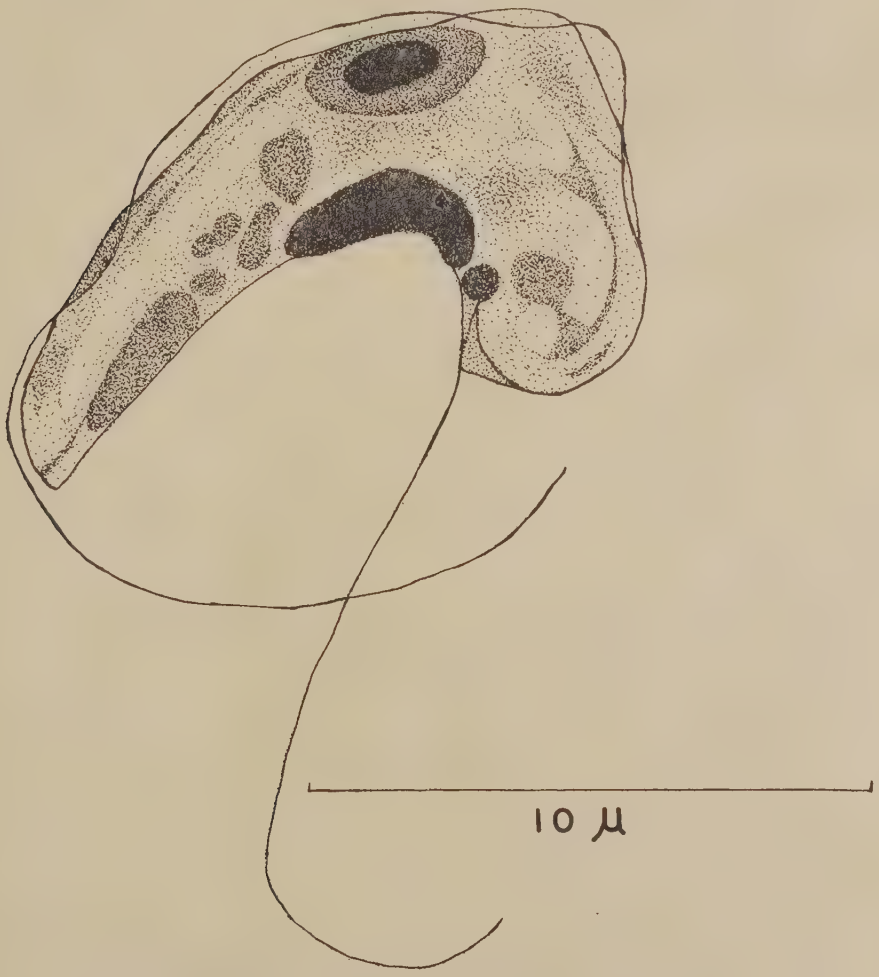


FIG. 2. *Cryptobia lynchi* n. sp. From photomicrograph of organism fixed in Schaudinn's and stained with iron-hematoxylin.

body width 2.46 (1.25–4.0) microns, s.d. 0.69; length of blepharoplast, 4.58 (2.0–9.0) microns, s.d. 0.78; width of blepharoplast, 0.5–2.0 microns; length of nucleus, 1.5–3.5 microns; width of nucleus, 1.0–2.5 microns; length of anterior flagellum, 16.05 (6.5–27.0) microns, s.d. 1.351; length of free portion of posterior flagellum, 8.96 (4.0–17.0) microns, s.d. 2.68; ratio of anterior flagellum to body length, 1.07 (0.40–1.95), s.d. 0.30; ratio of free portion of posterior flagellum to

body length, 0.61 (0.25–1.15), s.d. 0.18; ratio of anterior flagellum to free portion of posterior flagellum, 1.97 (0.6–3.7), s.d. 0.69.

Locality and Host: Soos Creek and Burns Creek, near Auburn, King County, Washington. Swamp Creek, near Kenmore, King County, Washington.

Host: Adult and fingerlings of *Oncorhynchus kisutch* (Walbaum).

Type Specimens: Holotypes and paratypes to be deposited in the United States National Museum.

Cryptobia lynchi new species

Figure 2

Specific diagnosis: (Average body dimensions based on the measurement of 106 specimens from 3 cottids.) Body length, 15.37 (8.0–25.0) microns, s.d. 3.47; body width, 3.49 (1.5–7.0) microns, s.d. 1.29; width of blepharoplast, 0.5–2.0 microns; length of blepharoplast, 4.46 (2.5–9.0) microns, s.d. 1.14; length of nucleus, 1.5–4.5 microns; width of nucleus, 1.0–3.0 microns; length of anterior flagellum, 13.30 (5.0–23.0) microns, s.d. 3.98; length of free portion of posterior flagellum, 8.37 (3.5–17.5) microns, s.d. 2.51; ratio of anterior flagellum to body length, 0.90 (.50–1.55), s.d. 0.23; ratio of free portion of posterior flagellum to body length, 0.57 (0.25–1.55), s.d. 0.23: ratio of anterior flagellum to free portion of posterior flagellum, 1.75 (0.6–3.5), s.d. 0.66.

Locality and Host: Soos Creek and Swamp Creek, Washington.

Hosts: Cottus rhotheus (Rosa Smith) and *Cottus aleuticus* Gilbert.

Type Specimens: Holotypes and paratypes to be deposited in the United States National Museum.

Because these hemoflagellates are reported from new hosts convention (Wenyon, 1926) condones the designation of these cryptobias as new and separate species.

TABLE 1.—*A comparison of the morphometric characters of* Cryptobia salmositica *and* Cryptobia lynchi

Character	Difference of means	Numbers of specimens compared	Significance of difference
Body length	0.43 microns	302	Not significant
Anterior flagellum	2.79 microns	184	Highly significant
Free portion of posterior flagellum	0.59 microns	174	Not significant
Anterior flagellum/body length	0.169	186	Highly significant
Free portion of posterior flagellum/body length	0.35	172	Not significant
Anterior flagellum/free portion of posterior flagellum	0.22	162	Significant

There is, however, some better justification to be found for the creation of new species to include these organisms. *Cryptobia salmositica* and *C. lynchi* differ from each other as well as from previously described cryptobias in several important morphometric characters. The average morphometric differences noted between *Cryptobia salmositica* and *C. lynchi* (Table 1) were tested for significance by the use of the "t" test for the difference of two means. (Snedecor, 1946.)

Although *Cryptobia salmositica* and *C. lynchi* are similar in length it is seen (Table 1) that the anterior flagellum of *C. salmositica* is longer than that of *C. lynchi*. The free portions of the posterior flagellum of both species are not significantly different in length. The relationship of the anterior flagellum to the body length differs in the two species with that of *C. salmositica* being greater. The two species of *Cryptobia* differ also in the relationship of the anterior flagellum to the free portion of the posterior flagellum with the ratio being greater in *C. salmositica*.

In addition, *Cryptobia salmositica* is clearly separate from those species of *Cryptobia* reported from the European trouts. *Cryptobia truttae* (Brumpt, 1906), and the presumably synonymous *C. valentini* (Gauthier, 1920) are described only from

living specimens found in the blood of the brown trout, *Salmo fario* taken in France. *C. salmositica,* on the other hand, is reported only from *Oncorhynchus kisutch* (Walbaum) a genus and species of fish which is native to certain areas of the North Pacific basin and which is furthermore, taxonomically and physiologically distinct from the European trout. It is, moreover, suspected that *C. salmositica* is specific to the genus *Oncorhynchus* for it was not found in the blood of five steelhead trout, *Salmo gairdnerii gairdnerii* (Richardson) which were in the Soos Creek trap at the same time as the infected salmon. Although all of the salmon present in the trap at that time were positive for this parasite, these trout did not have any hemoflagellates despite the fact that they were carrying the leech vector, *Piscicola salmositica* Meyer. These leeches when opened were full of the several developmental forms of the *Cryptobia.* The transmission of the flagellates to fish from the leech has been demonstrated by Brumpt (1906 a.b.) and Robertson (1911).

Morphological differences as well as host differences confirm the difference between *C. salmositica* and *C. truttae* (Brumpt). Unfortunately, Brumpt and

TABLE 2.—*The dimensions in microns of sixteen living unstained* Cryptobia salmositica *new species,* C. truttae (*Brumpt*) *and* C. valentini (*Gauthier*)

	C. salmositica	*C. truttae*	*C. valentini*
Length	13.0–25.5	20	30–40
Width	3–7		5–6
Anterior flagellum	16.05*	12	15
Free portion of posterior flagellum	8.96*	4	

* From stained specimens.

Gauthier described their hemoflagellates from an unspecified number of living unstained organisms and their descriptions are fragmentary and inadequate. From their descriptions, however, it can be seen, (Table 2), that *Cryptobia salmositica* is about the same length as *C. truttae* but is much smaller than the flagellate described by Gauthier (1920).

The living *Cryptobia salmositica* are about the same length as *C. truttae* (Brumpt) but have longer flagella. *C. salmositica* is shorter than *C. valentini* and its flagella are relatively larger. Accepting the mathematical proof of difference between *C. salmositica* and *C. lynchi,* then the much greater differences to be found between the flagellates of the silver salmon and the brown trout indicates that these also are separate species.

TABLE 3.—*The various bodily dimensions (in microns) and proportions of* Cryptobia lynchi *new species and* Cryptobia guernei (*Brumpt*)

Character	*Cryptobia lynchi*	*Cryptobia guernei*
Length	15.37 microns	34 microns
Anterior flagellum	13.30 "	16 "
Posterior flagellum	8.37 "	4 "
Blepharoplast	4.46 "	9 "
Nucleus	2.95 "	7 "
Anterior flagellum/body	.90	.46
Trailing portion of flagellum/body	.57	.12
Anterior flagellum/trailing portion of posterior flagellum	1.75	4.0

If one follows the above reasoning, *Cryptobia lynchi,* the parasite of the cottids is a species separate from *C. guernei* (Brumpt) which is described from *Cottus gobio,* a European cottid. Comparison of the measurements of these organisms, Table 3, indicates that *C. lynchi* is shorter with relatively longer flagella than *C.*

guernei. It is probable, therefore, that *Cryptobia lynchi* is a new species separate from *Cryptobia guernei* (Brumpt).

SUMMARY AND CONCLUSIONS

Two new species of *Cryptobia* have been reported from the blood of some fish native to the State of Washington. *Cryptobia salmositica* has been found in the blood of the silver salmon, *Oncorhynchus kisutch* (Walbaum) and *Cryptobia lynchi* from the blood of *Cottus rhotheus* (Rosa Smith) and *C. aleuticus* Gilbert. A careful comparison of the morphometric charactesr using valid statistical methods has indicated that these are two distinct species. Proven morphological differences as well as the new host records allow the author to describe these organisms as new species. Yet, it must be added that the literature survey preliminary to this manuscript made it evident that descriptions of many species of the genus *Cryptobia* are totally inadequate. Many of the previously described species, differ so little in size and morphology that it is impossible to differentiate them by their published descriptions. It seems worthwhile, therefore, to critically reexamine Keysselitz's (1906) contention that *Cryptobia* taken from various fish species resident in the same body of water are of the same species. The fish hosts are preyed upon indiscriminately by the same leech vectors and one leech could inoculate several species of fish with the same flagellate. The extremely slight variations in morphology reported by various authors are, therefore, probably invalid as diagnostic criteria and it is felt that many of the European species and perhaps the herein described species can be proven to be synonymous.

Although in this study, *Cryptobias* were examined from several salmonid and cottid hosts in an effort to determine specific differences, and the differences observed were proven significant, it is the author's opinion that complete and final proof of the validity of these two species must rest upon further experimental studies.

LITERATURE CITED

BRUMPT, É. 1906 Mode de transmission et évolution des trypanosomes des poissons; description de quelques espèces de trypanoplasmes des poissons d'eau douce. Trypanosome d'un crapaud africain. C. R. Soc. Biol. **60**: 162–164.

————1906 Expériences relatives au mode de transmission des trypanosomes et des trypanoplasmes par les hirundinées. C. R. Soc. Biol. **61**: 77–79.

GAUTHIER, M. 1920 Sur le "trypanosome" de la truite. C. R. Acad. Sci. **170**: 69–70.

KEYSSELITZ, G. 1906 Generations- und Wirtswechsel von *Trypanoplasma borreli*, Laveran und Mesnil. Arch. Protist. **7**: 1–74.

LAVERAN, A. AND MESNIL, F. 1901 Sur les flagellés à membrane ondulante des poissons (genres *Trypanosoma* Gruby et *Trypanoplasma* n. gen.). C. R. Acad. Sci. **133**: 670–675.

LEIDY, J. 1846 Description of a new genus and species of Entozoa. Proc. Acad. Nat. Sci., Philad. **3**: 100–101.

ROBERTSON, M. 1911 Transmission of flagellates living in the blood of fresh water fishes. Phil. Trans. Roy. Soc., Series B, **202**: 29–50.

SNEDECOR, G. W. 1946 Statistical Methods. The Iowa State College Press, Ames, Iowa.

WENYON, C. M. 1926 Protozoology, Volume II. Wm. Wood & Co., New York, N. Y.

NOTES ON THE TAXONOMY OF THE GENUS *COITOCAECUM* NICOLL, 1915 (DIGENEA: OPECOELIDAE)

PETER W. CROWCROFT*

William Nicoll (1915) described *Coitocaecum gymnophallum,* an interesting digenetic trematode in which the intestinal crura are fused posteriorly to form a continuous arch, from *Sparus australis,* taken off the coast of Queensland, Australia. Nicoll did not allocate *Coitocaecum* to a family of the DIGENEA, although he considered it to bear "a superficial resemblance to the ALLOCREADIIDAE." No additions were made to the genus for more than ten years, but thereafter species were reported from many parts of the globe. As far as I can learn the genus at present consists of the following species:—

TABLE 1

C. gymnophallum Nicoll, 1915	Queensland.
C. skrjabini Iwanitsky, 1928	Russia.
C. plagiorchis Ozaki, 1929 syn. *Ozakia plagiorchis*	Japan.
C. orthorchis Ozaki, 1929 syn. *Ozakia orthorchis*	Japan.
C. diplobulbosum Ozaki, 1929 syn. *Ozakia diplobulbosa*	Japan.
C. unibulbosum Ozaki, 1929 syn. *Ozakia unibulbosa*	Japan.
C. latum Ozaki, 1929 syn. *Ozakia lata*	Japan.
C. macrostomum Pigulevsky, 1931 syn. *Nicolla macrostoma*	Russia.
C. ovatum Pigulevsky, 1931 syn. *Nicolla ovata*	Russia.
C. testiobliquum Wisniewski, 1932	Yugoslavia.
C. proavitum Wisniewski, 1934	Yugoslavia.
C. anaspidis Hickman, 1934 syn. *Ozakia anaspidis*	Tasmania.
C. glandulosum Yamaguti, 1934	Japan.
C. species (unidentified) Wu, 1937	China.
C. species (unidentified) Dollfus, 1938	France.
C. acanthogobium Park, 1939 syn. *Ozakia acanthogobia*	Korea.
C. koreanum Park, 1939 syn. *Ozakia koreana*	Korea.
C. tropicum Manter, 1940 syn. *Ozakia tropica*	Galapagos Is.
C. wesuri Yamaguti, 1940 syn. *Ozakia wesuri*	Japan.
C. leptoscari Yamaguti, 1940 syn. *Ozakia leptoscari*	Japan.
C. parvum Crowcroft, 1944 syn. *Ozakia parva*	Tasmania.

Note: Synonyms of species described prior to Wisniewski (1934) by that author, those of later species by Manter (1947).

Many of the descriptions are accompanied by reviews of the literature and attempts to establish the status of the genus *Coitocaecum.* Discussion centers about the sub-family rank first proposed by Poche (1925), and the family rank proposed by Ozaki (1929). Opinions on these questions were expressed by Winfield (1929), Stunkard (1931), Woolcock (1935), Harshey (1937), Park (1939) and others. The modern trend, indicated by Manter (1940, 47) is to abandon the sub-family COITOCAECINAE Poche, and the family COITOCAECIDAE Ozaki, and to include *Coitocaecum* either in the sub-family OPECOELIINAE of the ALLOCREADIIDAE, or in the family OPECOELIDAE. The final elucidation of true inter-family relationships may well depend on such thorough embryological and larval studies as the work of Hunninen and Cable (1941), and Cable and Hunninen (1942), together with extended serological work initiated by Wilhelmi (1940). Our present limited knowledge of larval forms urges extreme caution in the evolution of working schemes for trematode identification at the higher level. A more pressing need is a measure of agreement on the evaluation of generic characters. Existing genera should be split only when *groups* of species possessing common similarities, and common differences from the other species in the genus, segregate within them. Premature splitting such

Received for publication, July 31, 1950.

* Now at Bureau of Animal Population, Oxford University.

as the scheme proposed by Wisniewski (1934) is sure to bring about great confusion in such a rapidly expanding group.

Wisniewski proposed to erect the genus *Nicolla* to accommodate *C. macrostomum* and *C. ovatum,* principally because of the median position of the cirrus sac and genital pore. This proposal seems premature and based upon insufficient evidence. With the addition of further species to the genus *Coitocaecum* a natural group of species possessing these and other characters in common may segregate and be placed in a separate genus to some useful purpose.

Into a different and perhaps more unfortunate category falls Wisniewski's proposal to set up the genus *Ozakia* for *C. plagiorchis,* with *C. orthorchis, C. diplobulbosum, C. unibulbosum,* and *C. latum.* Wisniewski was followed by Manter (1947), who transfered *C. anaspidis, C. glandulosum, C. tropicum, C. acanthogobium, C. koreanum, C. leptoscari, C. xesuri,* and *C. parvum* to *Ozakia.* This procedure leaves in *Coitocaecum* only the type species, *C. gymnophallum, C. testiobliquum* and *C. proavitum.* When Wisniewski's sketch illustrating the form of the cirrus sac in the genus *Coitocaecum sensu strictu* is compared with Nicoll's original figure of *C. gymnophallum* it is clear that Wisniewski identified the wall of the vesicula seminalis ("V.S." in Nicoll's figure) as a non-muscular cirrus sac containing cirrus, ejaculatory duct, and vesicula seminalis interna. This is born out by his generic diagnosis of *Coitocaecum.* With reference to the cirrus sac he stated—"Cirrusbeutel lang, kolbenförmig aus zwei Abschnitten bestehend; Vorderabschnitt schmal mit Ductus ejaculatorius und einzelnen Prostatadrüsenzellen, Hinterabschnitt breit mit Vesicula seminalis. Cirrusbeutel mit schwach entwickelten Muskelelementen . . ."

The Director of the School of Public Health and Tropical Medicine in the University of Sydney, with whom Nicoll's material is deposited, kindly sent me a specimen from the collection. He informed me that the label, which was in Nicoll's handwriting, bore the inscription—

No. 210. *Coitocaecum gymnophallum* Nic.
Host. *Sparus australis.*
Location. Intestine.
Locality. Cleveland Bay.
Collected by. W. Nicoll.
Date. 19. 6. 13.

This specimen was cleared and drawn in such detail as was possible (Fig. 1). It was then embedded and sectioned. The sections revealed the presence of a small membranous cirrus sac enclosing a short terminal portion of the male duct. The large seminal vesicle was clearly seen to be almost entirely external. At a later date I was able to visit Sydney and examine Nicoll's type specimen and sections. The staining of the whole-mount was now poorly differentiated, and little detail was visible in the forebody. The sections were completely bleached and extremely thin and distorted. The small cirrus sac could not be discerned but it was clear that the seminal vesicle was not enclosed by another wall.

EXPLANATION OF FIGURES

Abbreviations: a.c., acetabulum; c.s., cirrus sac; e., eggs; ex., excretory vesicle; gl., pharyngeal glands; g.p., genital pore; int., intestine; o.s., oral sucker; ov., ovary; ph. pharynx; s.v., seminal vesicle; tes., testis; vit., vitelline follicles; y.v., yolk reservoir.

FIG. 1. *Coitocaecum gymnophallum* Nicoll, 1915.
FIGS. 2–5. *Coitocaecum parvum* Crowcroft, 1945, showing morphological variations.

Although Nicoll (1915), at the conclusion of his description referred to the "absence of a cirrus pouch . . .," earlier in his description he states—"There is no *true* cirrus pouch." (My italics.) Possibly Nicoll observed the presence of the small membranous sac which we would refer to as a membranous cirrus "sac," but he did not consider it, in the light of contemporary usage to constitute a true cirrus "pouch."

Thus the condition of the cirrus sac in *C. gymnophallum is* seen to fall within Wisniewski's diagnosis of *Ozakia*—"Cirrusbeutel kurz, überschreitet nie den Darmschenkel, mit oder ohne Muskeln; stark reduziert, zusammengesetzt aus Cirrus (wenn vorhanden), Pars prostatica (wenn vorhanden), Ductus ejaculatorius und vesicula seminalis interna. . . ." Accordingly the genus *Ozakia* must fall and the species transferred to it by Wisniewski (1934) and Manter (1947) are transferred back to *Coitocaecum*. If *C. ovatum, C. macrostomum* and *C. skrjabini* possess a muscular cirrus sac enclosing the seminal vesicle, and if further species possessing this character are described, it may be useful to set up a genus to accommodate them. In this connection I am unable to discuss, in anticipation of its publication, a new proposal for the splitting of *Coitocaecum* which has been worked out by Professor Robert Ph. Dollfus.

Yamaguti (1934) when describing *C. glandulosum,* which closely resembles *C. gymnophallum,* mentioned the presence of prominent gland cells in the vicinity of the anterior intestinal arch in his species, which he suggested may have been overlooked by Nicoll. When examining Nicoll's type specimen I observed apparently similar structures, proving Yamaguti's surmise correct. These gland cells were not present in the smaller specimen of *C. gymnophallum* which I sectioned, although gland cells were very prominent about the prepharynx (Fig. 1). It may be that the gland cells about the anterior intestinal arch are a morphological manifestation of physiological changes brought about upon the attainment of larger size. With regard to the reported difference in egg size in the two species, we are not yet decided upon the divergence in egg size to be tolerated within a species, nor aware of the effects of growth and development of the one species within different hosts. There is also the possibility that egg size changes with the age of the fluke and the number of eggs produced. It is interesting to note that the eggs of a small specimen of *C. gymnophallum,* approximately 2.5 mm. long, measure 0.088 mm., those of Nicoll's type, 3 mm. long, measure 0.081–0.084 mm., and those of Yamaguti's specimens of *C. glandulosum* 3.65 mm. long measure 0.063–0.07 mm. If the two species are identical an inverse proportion between body length and egg size is indicated, although it remains to be shown whether or not these egg size differences are constant throughout a large number of specimens. Until more is known concerning the diagnostic value of egg size, it seems desirable to retain *C. glandulosum* as a valid species.

Yamaguti (1940) regarded *C. diplobulbosum* and *C. unibulbosum* as identical. Ozaki (1929) separated them principally on the shape and relative size of the pharynx. Yamaguti pointed out that these characters are variable according to the state of contraction at fixation. The fact that the two species are reported simultaneously from the same fish host indicates the possibility of arbitrary division of the material based upon size and fixation variations. Ozaki, however, considered the occurrence of five specimens in all of which the pharynx is relatively smaller than in *C. uni-*

bulbosum, and possesses a constriction near the posterior end as sufficient evidence of speciation. I have never observed a constricted pharynx as illustrated by Ozaki for *C. diplobulbosum* to result during fixation, and prefer to accept this species as valid until more evidence is obtainable to the contrary.

Some time after describing *C. parvum* (Crowcroft 1945), I had occasion to prepare over sixty whole-mounts of this species under uniform conditions of fixation and staining. The method used was to mount the fluke in saline and irrigate with 90% alcohol while maintaining just sufficient pressure on the cover-glass to prevent undue contraction. From examination of flukes before and after fixation I have come to the conclusion that although the breadth is increased slightly, very little distortion or organic displacement results from this method. After hardening they were stained overnight in ammonium-alum carmine solution and differentiated with weak acid-alcohol. As a wider knowledge of natural (and artificial) variation is prerequisite to the avoidance of the setting up of invalid species, several variants, not considered to result from the fixation process are illustrated (Figs. 2–5). In one specimen only was there significant displacement, the genital pore being displaced from the usual position on the left side, to a median position within the anterior intestinal arch. The principal dimensions of the forms shown in Figures 2–5 are given in the following table.

TABLE 2

	Fig. 2.	Fig. 3.	Fig. 4.	Fig. 5.	*C. parvum*
Length:	1.60	2.05	2.24	2.14	0.57–1.8
Breadth:	0.78	0.84	0.79	0.53	0.34
Forebody:	0.38	0.58	0.66	0.47	. . .
Oral sucker:	0.25	0.20	0.18 × 0.20	0.14 × 0.17	0.09
Acetabulum:	0.26 × 0.32	0.29 × 0.42	0.31 × 0.35	0.23 × 0.27	0.14 × 0.19
Eggs:	0.056 × 0.032	0.064 × 0.040	0.068 × 0.040	0.064 × 0.036	0.060–0.076 × 0.032–0.040
Position of Testes:	Tandem.	Oblique.	None.	Tandem.	Tandem or oblique.

In the specimen shown in Figure 2 breakdown of the yolk follicles is proceeding. It is accompanied by, or associated with, swelling of the peripheral regions of the body or shrinkage of the intestine. The seminal vesicle could not be seen, and the body contained numerous large darkly staining cells which are much less frequent in normal individuals.

In view of the absence of the main diagnostic feature of *Coitocaecum* the form shown in Figure 3 would apparently belong in another genus. I consider the unequal length of the crura, coupled with the oblique form of the posterior end of the body, to suggest previous injury or physiological upset, and that this specimen is an aberrant form of *C. parvum*. Perhaps the abnormal condition is also expressed in the form of the posterior testis.

The absence of one or both of the testes is not unknown in the DIGENEA and may prove of more frequent occurrence than would appear from existing records. Figure 4 illustrates a form in which the testes are completely atrophied. A little non-staining debris alone indicates their former position. The seminal vesicle has shrunk and retreated to a median position in front of the acetabulum. The size and form of the seminal vesicle are quite variable in this species. Other specimens show the extension of the vesicle posteriorly along the left side of the acetabulum culminating in the large club-shaped vesicle shown in Figure 5. Intermediate forms exist, linking this latter elongate form with the usual broader form with oblique testes figured in the original description of the species.

While collecting *C. parvum* from *Galaxias attenuatus* Jenyns, several specimens of an *Opecoelus* sp. were found. The determination of the presence or absence of an anus cannot always be made with certainty from whole-mounts, but a useful difference in the physiology of the two genera results in the differential absorption of ammonium-alum carmine. The *Opecoelus* sp. specimens were readily separated out by means of this differential color reaction with the stain.

References

Cable, R. M. and Hunninen, A. V. 1942 Studies on *Deropristis inflata* (Molin); its life history and affinities to trematodes of the family Acanthocolpidae. Biol. Bull. **82:** 292–312.

Crowcroft, P. W. 1945 New trematodes from Tasmanian Fishes. Pap. Proc. Roy. Soc. Tasm. 1944 (45): 61–69.

Harshey, K. R. 1937 On two new trematodes of the genus *Opegaster* Ozaki, with a systematic discussion on the families Opecoelidae Ozaki, 1925 and Coitocaecidae Ozaki, 1928. Proc. Indian Acad. Sci. **18:** 64–75.

Hunninen, A. V. and Cable, R. M. 1941 Studies on the life history of *Anisoporus manteri* Hunninen and Cable, 1940. (Trematoda-Allocreadiidae). Biol. Bull. **80:** 415–28.

Manter, H. W. 1940 Digenetic trematodes of fishes from the Galapagos Islands and the neighboring Pacific. Rep. Coll. A. Hancock Pacific Exped. 1932–38, 2 (14): 329–497.

———1947 The digenetic trematodes of marine fishes of Tortugas, Florida. Amer. Midl. Nat. **38:** 257–416.

Nicoll, W. 1915 The trematode parasites of North Queensland. III. Parasites of fishes. Parasitol. **8:** 22–40.

Ozaki, Y. 1929 Notes on Coitocaecidae, a new trematode family. Annot. Zool. Japon. **12:** 75–90.

Park, J. T. 1939 Trematodes of fishes from Tyosen. III. Some new trematodes of the family Allocreadiidae Stossich, 1904 and the genus *Macrolecithus* Hasegawa and Ozaki, 1926. Keijo J. Med. **10:** 52–61.

Poche, F. 1925 Das System der Platodaria. Arch. Naturg. **91:** 1–451.

Stunkard, H. W. 1931 Further observations on the occurrence of anal openings in digenetic trematodes. Zeit. Parasitenk. **3:** 713–725.

Wilhelmi, R. W. 1940 Serological reactions and species specificity of some helminths. Biol. Bull. **79:** 64–90.

Winfield, G. S. 1929 *Pleisocreadium typicum*, a new trematode from *Amia calva*. J. Parasit. **16:** 81–87.

Wisniewski, L. W. 1934 Beitrag zur Systematik der Coitocaecidae (Trematoda). *Nicolla* g. n., *Ozakia* g. n., *Coitocaecum proavitum* sp. n. Mem. Acad. Polon. Sci. Lett., ser. B, **6:** 27–41.

Woolcock, V. 1935 Digenetic trematodes from some Australian fishes. Parasitol. **27:** 309–331.

Yamaguti, S. 1934 Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes I. Jap. J. Zool. **5:** 249–541.

———1940 Studies on the helminth fauna of Japan. Part 31. Trematodes of Fishes, VII. Jap. J. Zool. **9:** 35–111.

NOTES ON THE SPECIES OF *CAPILLARIA* ZEDER, 1800 KNOWN FROM GALLINACEOUS BIRDS

Holger Madsen

The species of the nematode genus, *Capillaria* Zeder, 1800, present many taxonomic difficulties. The most important of more recent studies regarding species in birds are those of Orosz (1931), Morgan (1932), Freitas & Almeida (1935a–b), Cram (1936) and Read (1949). Based upon large collections from gallinaceous and anatine game birds the present author (1945) tried to clear up the difficulties and confusions in the very scattered literature on these species. Since then I have found several new features especially concerning the species in gallinaceous birds. I consider it, therefore, of some use to summarize the results, and give a revised list of the species from these birds.

The genus *Capillaria* comprises a large number of species. It would, therefore, be useful if it could be split into several genera. Some attempts have been made in this respect, beginning with Dujardin (1845). Baylis (1931) showed that the attempts up to that time had been futile, and this point of view was accepted by the above mentioned authors. Recently, López-Neyra (1947), in a very comprehensive paper, revived some of the genera discarded by Baylis (1931), and erected some new ones. As principal characters for segregating the genera he used the ratio between the length of the esophagus, and of the entire body, and the presence or absence of spines on the spicule sheath. It is already doubtful to use differences in size, and even more ratios taxonomically. This is the more objectionable since he did not compute this ratio on the individual ratios, but took the first and last figure in the range of the length of the esophagus and of the posterior part of the body, respectively, computing the range of the ratio on these figures. For instance, in a table, p. 43, quoting measurements by Wehr (1939), on *Capillaria obsignata,* he found a range of the said ratio of 1.23–1.59; whereas the range, computed on the indivudal measurements is actually 1.20–1.88. For the main grouping in his key, p. 46–47, he used the feature, whether the esophagus constitutes less or more than one-third of the total length, and as subgrouping in both groups, spiny or non-spiny spicule sheath. If this key is strictly followed it should be possible to place, e.g. my specimens of *Capillaria caudinflata* in no less than four of his genera! (cf. Holger Madsen (1945, p. 15 and fig. 4)). I cannot, therefore, find an attempt at erecting subgenera or genera justified, when based upon the characters known at present, and prefer to adopt the opinion of Baylis.

In the synopsis given by López-Neyra, and by me in 1945, species described by Yamaguti (1941) and Johnston & Mawson (1941, 1945) are not included.

In the following list the new points of view will be given under the heading of the individual species.

Capillaria contorta (Creplin, 1839) Travassos, 1915.

Synonyms: (*Trichosoma contortum* Creplin, 1839); *T. annulatum* Molin, 1858; *T. longicolle* Rudolphi, 1819 of Stossich, 1890, p. p; *T. strumosum* Reibisch, 1893;

Received for publication, April 25, 1950.

Jagtfondens vildtbiologiske Undersøgelser, Nørregade 10, Copenhagen, K, Denmark

T. delicatissimum Perroncito & Tomiolo, 1900; *Capillaria perforans* Kotlán & Orosz, 1931; *C. lophortygis* Baylis, 1934; *C. vanelli* Yamaguti, 1935; *Eucoleus raillieti* López-Neyra, 1947.

As will be seen in the above list, I consider both *Capillaria perforans* and *Capillaria annulata* as synonyms.

Cram (1936, p. 22) already expressed doubt as to whether *Capillaria perforans* is a distinct species, a view I accepted (1945, p. 47) without having seen the papers by Orosz (1931) and Vaz (1939), which were not available to me. Now, that I have seen their figures and descriptions, I find my opinion unchanged. The general morphology is just the same: the shape of the tail of the male, the spicule (when discernible) and spicule sheath, the eggs, and the tail of the female. The only difference is the size of the body and the spicule. Since this "species" apparently has always been found associated with severe pathological conditions, it seems reasonable to consider it only a modification of *Capillaria contorta,* which is caused by favorable conditions, the nature of which, it is true, is unknown.

Because of the cuticular inflation around the mouth, *Capillaria annulata,* since 1858, has been recognized as a distinct species, partly under the names of *C. strumosa* and *C. delicatissimum.* The inflation is very variable in appearance and size, as are the cuticular swellings along the neck [compare the illustrations by Molin (1861), Reibisch (1893), Ciurea (1914), Tubangui (1927), Orosz (1931), Freitas & Almeida (1935) and Todd (1946)]. It is generally stated that both the inflation and the swellings are usually missing in young specimens, and may collapse or shrink in adults (Cram, 1936, p. 4)). On the other hand, according to the same authors, the morphology of the other parts of the worms cannot be distinguished from those of *Capillaria contorta.* For instance, the eggs are identical, as is the shape of the vulva, the tail of the female and the male.

In specimens from the turkey Wehr (1937) indicated apparent macroscopical differences from *Capillaria contorta.* These can readily be explained as variations in size. By the courtesy of Dr. Wehr I received from the U.S.A. some specimens labelled *Capillaria annulata.* Most of these are unquestionably *Capillaria contorta.* One female specimen, however, possesses cuticular swellings, but otherwise it is a typical *Capillaria contorta.* Quite recently I found in the esophagus and the crop of a pheasant in Denmark several specimens, of which some few, situated near the mouth, were small, and had cuticular swellings; but in every other respect they were typical *Capillaria contorta.*

One further argument for the identity I find striking, as it inclined my previous doubts as to the validity of the species on purely morphological grounds, to ripen into a conviction: the previously puzzling absence of *Capillaria contorta* in the domestic fowl, when the species is of common occurrence in a large number of other gallinaceous birds, to say nothing of the other, unrelated, hosts. Thus, in my opinion, we find in the two "species," *Capillaria perforans* and *Capillaria annulata* only structural modifications, which in the first case may be due to conditions favorable to the worm, and in the other unfavorable conditions.

Objections might be raised against the above view, that *"C. annulata"* is a synonym of *C. contorta.* It is indicated that eggs of *C. contorta* average about 53 μ in length, whereas those of *"C. annulata"* average about 65 μ. But such differences in size are not necessarily of taxonomic value. For instance, in my own material

of *C. contorta* (cp. Holger Madsen, 1945, p. 39, table 5) it can be demonstrated that at any rate the male specimens from ducks and pheasants, respectively, present statistically significant differences in length. Furthermore, according to the literature, the range of size of the eggs of *C. contorta* is 46–70 μ, whereas that of *"C. annulata"* is 54–66 μ (cf. Madsen, 1945, p. 97, table 12), thus being within the range of *C. contorta.* Even the fact that eggs from specimens of *"C. annulata"* from chickens and turkeys do not differ in average length or in any other respect supports my view. If *"C. annulata"* is only a modification of *C. contorta,* somehow caused by factors in the host, it is not surprising that the forms are alike also in other features than in the swellings.

It is sometimes stated that, in older infections, a cephalic swelling, or some trace of it, invariably occurs in specimens of *"C. annulata."* This seems to me to be a circle inference, since it is only *because* of these swellings that the specimens are called *"C. annulata."* *No other difference at all in the morphology can be found* either in the descriptions in the literature or in my own observations.

The time of the appearance of the swellings is not uniform. In some larvae they may occur as early as 10 days after experimental hosts have been infected, in other cases they have not developed even at the time when egg production commences. This, I find, suggests some influence from part of the host.

It might be objected, too, that if the presence of a swelling does not indicate a specific difference, how can the common occurrence in turkeys of worms without swellings be explained, when in chickens only worms having a swelling are to be found? This, again, is readily explained from the same assumption that chickens just react in some other way against the worm than turkeys do. My above cited find of *"C. annulata"* only in the uppermost part of the esophagus of a pheasant points in the same direction.

Finally, Wehr (1936) and Zucchero (1942) were able to demonstrate that *"C. annulata"* requires earthworms as intermediate hosts, whereas *C. contorta,* according to Cram (1936) does not. Cram made several attempts of experimental infestations, but they were not always successful. As already suggested by Cram (1936, p. 19) the explanation is probably that we have here a case similar to that of *Syngamus trachea,* in which an intermediate host may or may not be involved, and that infection is established more easily with an intermediate host.

It must be admitted, however, that it would be very desirable to feed eggs of *C. contorta* to earthworms, and then these again to chickens. A positive result would then give an exact proof.

"Capillaria perforans" was found in the turkey in Central Europe, and in the same host, as well as in *Numida meleagris, Numida ptylorhyncha,* and pheasant, in South America. *"Capillaria annulata"* was found all over the world in poultry, and in *Meleagris* and *Numida.* Further it is rather common in various game birds, namely *Lyrurus tetrix* and *Tetrao urogallus* in Europe, in *Bonasa umbellus* and *Colinus virginianus* in the U.S.A., and in both areas in pheasants and partridges (see Graham (1935)). In all of the above hosts, with the exception of domestic hen and the species of *Numida,* the typical *Capillaria contorta* was also found.

López-Neyra's new species, *Eucoleus raillieti,* is based upon the specimens (in the literature) from ducks. All these are undoubtedly *Capillaria contorta.* Besides, in the hosts mentioned in my paper of 1945, I have later seen typical *C. contorta* in the rook (*Corvus frugilegus*) in Denmark.

Occurrence[1]: The esophagus of fowls and other domestic gallinaceous birds; in numerous gallinaceous and anatine game birds; in charadriiform and passerine birds. Widely distributed.

Capillaria uropapillata Freitas & Almeida, 1935.

In this species only the females are known. In its general morphology and dimensions, it resembles *C. contorta* very much. Unfortunately, the eggs are not figured. This species seems to differ in the slit shaped vulva (this is circular to oval in *C. contorta*) and in the small papillae on the tail. Such papillae I have never seen in my abundant material of *C. contorta.*

Occurrence: The esophagus of *Phasianus colchicus,* Brazil.

Capillaria caudinflata (Molin, 1858) Wawilowa, 1926.

Synonyms: *Gordius* (Drahtwurm) des Huhns, Goeze, 1782 (?); *Filaria gallinae* Schrank, 1788 (?); *Capillaria semiteres* Zeder, 1803, p.p. (?); *Hamularia nodulosa* Rudolphi, 1809, p.p. (?); *Trichosoma columbae* Rudolphi, 1819 (?); *Trichosoma longicolle* Rudolphi, 1819 of Mehlis, 1831; *Calodium tenue* Dujardin, 1845) (?); (*C. caudinflatum* Molin, 1858); *Trichosoma gallinum* Kowalewski, 1894; *T. papillosum Blome,* 1909; *T. papilligera* Railliet & Henry, 1911; *T. meleagris-gallopavo* Barile, 1812; *Capillaria dujardini* Travassos, 1914 (?), non 1915; *Capillaria meleagris* (Barile, 1912) of Travassos, 1915; *C. blomei* Travassos, 1915; *C. caudinflata* (Molin, 1858) var. *balthica* Otte, 1928; *C. bursata* Freitas & Almeida, 1934; *C. columbae* (Rudolphi, 1819) var. *sturni* Cannon, 1939; *C. longicollis* (Mehlis, 1831) of Holger Madsen, 1945; *C. gallinae* (Goeze, 1782) of López-Neyra, 1947.

Read (1949) demonstrated that my "conservation" (Holger Madsen, 1945, p. 11) of the name *Capillaria longicollis* for this species is not allowable according to the rules of nomenclature. Orosz (1931) and Morehouse (1944), gave good reasons for using the name *C. caudinflata.* Their papers were not available to me when I wrote my paper.

As pointed out by me in 1945, and recognized by Read (1949), the *Trichosoma columbae* Rudolphi, 1819 (whose specimens were later described by Dujardin as *Calodium tenue*) can not be the current *"C. columbae"* (= *C. obsignata,* see below). Since Dujardin described the occurrence of a membranous appendage at the vulva, and since *C. caudinflata* also occurs in pigeons, *Trichosoma columbae* is probably a synonym of *C. caudinflata.*

López-Neyra (1947) treated this species under three names. His illustrations clearly show that the specimens he has seen himself belong to the species under consideration. He considers the correct name of this species to be *Capillaria gallinae* (Goeze, 1782) López-Neyra, 1947. As will be seen in the list of synonyms, Goeze did not name his species in a Linnean sense. The name was given by Schrank (1788). He, therefore, is the author if Goeze's worm is accepted as being identical with the species in question. It must be admitted that the "Gordius from the hen" is probably the same as the species treated here, since it is a species of *Capillaria* found in the small intestine, furthermore being of a size far exceeding that which is generally found in the other small-intestinal worm, *C. obsignata,* occurring in fowls,

[1] Particulars regarding host occurrence of the species here treated may be found in my paper of 1945.

and it is expressly stated by Goeze (p. 126) that the figured worm was found together with specimens of *Ascaridia*. On the other hand, the morphological details given in the description and illustrations do not allow a safe determination of the species. Moreover, the shape of the tail as figured (Goeze, pl. VII B, fig. 9) differs from that of *Capillaria caudinflata*, and the prevulvar part of the body (as judging from the distribution of the eggs) seems to be shorter than is generally the case in this species. In both respects there are greater similarity to the cropworm, *Capillaria contorta*. Owing to these uncertainties in the identification is seems reasonable to keep the first name which is connected with a *certain*, recognizable description, which is the *Calodium caudinflatum* of Molin (1858), who furthermore in 1861 gave a figure, which unquestionably illustrates the species treated here.

In 1945 (p. 21) I expressed doubt as to the validity of *Capillaria bursata*. The illustrations given by Todd (1946) have strengthened my suspicion, the main reason being that the picture of the egg is identical with that of *Capillaria caudinflata*. The vulva, it is true, seems to be rather different. On the other hand, I have myself seen specimens with a series of cuticular bosses like those figured by Todd, and at the same time without a vulvar appendage, this apparently having been broken off; and the specimen by me called *Capillaria* cf. *bursata* from a pheasant probably belongs here, too. (cf. Holger Madsen (1945, p. 21, fig. 6, f.).) The curious appearance of the vulva is, therefore, after all due to an abnormality. The paper by Todd (1947), in which he gives comments upon, and measurements of *"Capillaria bursata,"* has not changed my view. It is striking that his measurements lie, practically, within the range found by me in *Capillaria caudinflata*, with an advantage of the higher figures. This corresponds well with the fact that my above mentioned specimens, being without vulvar appendage, and with cuticular bosses, are large ones. Recently I have seen female specimens of *"C. bursata"* submitted to me by Todd. These were quite identical with my above mentioned specimens, which had lost the vulvar appendage. Further knowledge of the morphology of the spicule is needed.

The vulvar appendage of *Capillaria caudinflata*, as figured by Todd (1946) also is not normal. Altogether, the vulvar appendage in this species is very often found deformed in the preparations.

Occurrence: Small intestine of fowls. In addition in numerous gallinaceous game birds (not in the U.S.A.!), in species of *Columba*, in *Otis tetrax, Sturnus vulgaris; Turdus migratorius* (Read (1950)); domestic goose, muscovy duck (Owen, Wales, unpublished), and (experimentally) in the house sparrow. Widely distributed.

Capillaria obsignata Holger Madsen, 1945.

Synonyms: *Trichosomum tenuissimum* Diesing, 1851 of Eberth, 1863, nec *Calodium tenue* Dujardin, 1845, nec *Trichosomum* (*Calodium*) *tenuissimum* Diesing, 1851 (= *Trichosoma columbae* Rudolphi, 1819 = *Capillaria caudinflata* (Molin, 1858) (?)), nec *Trichocephalus tenuissimus* Rudolphi, 1803 (= *Capillaria tenuissima* (Rudolphi, 1803) = *Capillaria strigis* (Froelich, 1802) López-Neyra, 1947) (?)); *Trichosoma columbae* Rudolphi, 1819 of Stossich, 1895, p.p; *Capillaria dujardini* Travassos, 1915, non 1914; *Capillaria dujardini* Travassos, 1915 of Holger Madsen, 1945.

This species is the current *"Capillaria columbae."* Read (1949) demonstrated

that the name should be as here used. López-Neyra's (1947) own specimens of *"C. columbae"* belong to the present species, as his illustrations prove.

Occurrence: The small intestine of fowls and other domestic gallinaceous birds, domestic pigeon, widely distributed. In *Turdus migratorius* in the U.S.A., in *Perdix perdix* and *Phasianus colchicus* in Denmark.

Capillaria tiaras Holger Madsen, 1945.

This species might prove to be identical with the insufficiently described *Capillaria alaudae* (Rudolphi, 1819), or some other of the little known species from passerine birds.

Occurrence: the small intestine of partridge chick in Denmark.

Capillaria anatis (Schrank, 1790) Travassos, 1915.

Synonyms: (*Trichocephalus anatis* Schrank, 1790); *Filaria phasiani* Froelich, 1791 (?); *Linguatula unilinguis* Schrank, 1796 (?); *Filaria tetricis* Froelich, 1802 (?); *Capillaria tumida* Zeder, 1803; *C. semiteres* Zeder, 1803, p.p. (?); *Hamularia nodulosa* Rudolphi, 1809, p.p. (?); *Trichocephalus capillaris* Rudolphi, 1809, p.p.; *Trichosoma brevicolle* Rudolphi, 1819, p.p.; nec *Trichosoma brevicolle* Rudolphi, 1819 of Eberth, 1863 (= *Capillaria mergi* Holger Madsen, 1945); *T. longicolle* Rudolphi, 1819; *T. collare* v. Linstow, 1873; *T. retusum* Railliet, 1895; *T. dubium* Kowalewski, 1894; *Capillaria uruguayensis* Calzada, 1937 (?); nec *C. anatis* (Schrank, 1790) of Gorschkov, 1937 (= *C. anseris* Holger Madsen, 1945); *Echinocoleus collare* (v. Linstow, 1873) of López-Neyra, 1947.

Trichosoma longicolle Rudolphi, 1819 was found in the ceca of fowls. No recognizable description was given. It seems reasonable to assume that it is identical with the generally occurring cecal *"Capillaria collaris"* (= *"C. retusa"*).

Mr. Richard Owen (Aberystwyth, Great Britain) recently turned my attention to the probable identity of *Capillaria collaris* and *Capillaria anatis*. The only difference between the two "species" seems to be the unevenness of the surface of the eggs, as will be seen, e.g. from the illustrations and measurements given in my paper of 1945. By reexamining my preparations of *"Capillaria collaris"* it turned out that this feature, only more inconspicuously, could be found. This is also seen in the figures in Tubangui (1927) and Morgan (1932). The unevenness of the surface of the egg was also quite distinct in some specimens from fowls in Denmark.

Capillaria uruguayensis Calzada, 1937 can not with certainty be segregated from *C. anatis*. If the eggs had been figured more accurately the question would have been easier to decide.

Occurrence: Mostly the ceca of domestic gallinaceous and anatine birds, widely distributed; gallinaceous game birds in Europe.

Capillaria phasianina Kotlán, 1940.

Synonyms: *Trichosoma longicolle* Rudolphi, 1819 of Parona, 1886, p.p. (?); *Capillaria collaris* (v. Linstow, 1873) of Holger Madsen, 1941, p.p.; *C. cadovulvata* Holger Madsen, 1945.

Only recently have I seen the paper by Kotlán (1940). He described as new a species of *Capillaria,* which he found in the ceca of pheasants in Hungary. His illustrations show that he had the same species before him, which I have in my col-

lections. Although several details, especially concerning the features of the spicule, are not clear from the description and pictures given, there is still such good agreement that the identity is without doubt: the lateral lobes at the tail end of the male, the spines on the spicule sheath; in the female the cuticular appendage on the vulva, within which an egg is often seen, the swelling of the body behind the vulva, with a glandular structure; the shape of the eggs is identical. The lengths of the body lie within the limits of variation found by me, as does his figures of the egg's size. Only one striking difference, in the length of the spicules, seems to exist. Whereas I found a range of 1.77–2.66 mm., Kotlán gave the length at 190–200 μ. So small a length of the spicule has only rarely been found in species of *Capillaria* in birds, and seems quite improbable, already at a first glance at the figure of the caudal end of the male, given by Kotlán. The breadth of the body at the caudal end is given as 50 μ. In using this distance as a measuring unit, it appears that the length of the protruded spicule sheath is already 129 μ, and it is apparent that only a very small part of the spicule proper is figured. It is, therefore, in my opinion without doubt that there is a mistake here, which may readily be explained in assuming that the figures are given ten times too small. In adding a zero, a range of 1.90–2.00 mm. is obtained, which lies within the range found by me.

A comparison with the species of *Capillaria* from birds, the descriptions of which were not accessible to me during the war, shows that the present form bears some resemblance to *Capillaria graucalina* Johnston & Mawson, 1941, from *Coracina* (*Graucalus*) *novae-hollandiae,* the location in the host not being mentioned. The tail of the male has a similar shape, but the spicule sheath seemingly bears no spines. Furthermore, the spicule is cylindrical, which is in contrast to the triangular shape, being found in transverse section in the present species. The female has a vulvar appendage, and a similar constriction just behind the vulva, as here, but the shape of the appendage, and that of the egg is different, judging from the rather indistinct illustration given.

Occurrence: the ceca of *Phasianus colchicus* (Denmark, Hungary), *Perdix perdix* (Denmark; England (Clapham (1949)) and *Chrysolophus pictus* (Denmark, confined).

Capillaria vazi Freitas, 1933.

This species bears a close resemblance to *Capillaria phasianina,* but is quite distinct, as pointed out in my paper of 1945.

Occurrence: In an unknown section of the intestine in *Odonthophora capueira,* Brazil.

Capillaria montevidensis Calzada, 1937.

This is clearly a distinct species.

Occurrence: The ceca of fowls, Uruguay.

Capillaria exilis (Dujardin, 1845) Travassos, 1915.

Synonyms: *Trichosomum exile* Dujardin, 1845; nec *Liniscus exilis* Dujardin, 1845 (= *Capillaria incrassata* (Diesing, 1851)); nec *Trichosomum exile* Dujardin, 1845 of Eberth, 1863 (= *Capillaria rasilis* Holger Madsen, 1945).

Baylis (1939) found this species in a pheasant in England; also in some thrushes and the European starling.

As seen in the list of synonyms, two species were described under this name from the same host, viz. some thrushes, one seen by Dujardin (1845) (recently having been redescribed by López-Neyra (1947)), and one by Eberth (1863). This latter I gave the name *Capillaria rasilis*.

The specimen described by Eberth differs in the shape of the male's tail, in having no cuticular membrane on this structure, and especially in that the spicule is triangular in cross section, whereas in Dujardin's species it is circular.

SUMMARY

A revised list of ten species of *Capillaria* hitherto known in gallinaceous birds is given. It is demonstrated that the attempt by López-Neyra (1947) at a subdivision into several genera of the genus *Capillaria* Zeder, 1800 is not successful. The identity of several species previously considered separate is ascertained: "*Capillaria annulata* (Molin, 1858)," "*C. raillieti* López-Neyra, 1947" = *C. contorta* (Creplin, 1839). "*C. bursata* Freitas & Almeida, 1934" = *C. caudinflata* (Molin, 1858). "*C. collaris* (v. Linstow, 1873)" = *C. anatis* (Schrank, 1790). As a new synonym is further given "*C. cadovulvata* Holger Madsen, 1945" = *C. phasianina* Kotlán, 1940.

REFERENCES

BAYLIS, H. A. 1931 On the structure and relationship of the nematode, *Capillaria* (*Hepaticola*) *hepatica* (Bancroft). Parasitol. **23**: 533–543.

———1939 Further records of parasitic worms from British vertebrates. Ann. Mag. Nat. Hist. (11), **4**: 473–498.

CALZADA, V. 1937 Nuevos nematodes. Dos nuevos especies del genero *Capillaria* Zeder. *Capillaria montevidensis—Capillaria uruguayensis*. Parasitos de *Gallus dom.* L. Anal. Fac. Vet. **3**: 73–79.

CIUREA, J. 1914 Nematoden aus dem Pharynx und Oesophagus des Haushuhnes. Z. Infek. Haustiere **15**: 49–60.

CLAPHAM, P. A. 1949 On *Capillaria cadovulvata*, pathogenic to *Perdix perdix*. J. Helminth. **23**: 1–2, 69–70.

CRAM, E. B. 1936 Species of *Capillaria* in the upper digestive tract of birds. Techn. Bull. Dep. Agric. No. 516: 1–27.

DUJARDIN, F. 1845 Histoire naturelle des helminthes ou vers intestineaux. Paris.

EBERTH, C. J. 1863 Untersuchungen über Nematoden. Leipzig.

FREITAS, J. F. T. DE AND ALMEIDA, L. DE 1935a Sobre os nematoda *Capillariinae* parasitas de esophago e papo de aves. Mem. Inst. Oswaldo Cruz **30**: 123–156.

——— AND ———1935b O genero *Capillaria* Zeder, 1800 (Nematoda-Trichuroidea) e os capillarioses nas aves domesticas. Rev. Dept. Nac. Prod. Anim. **2**: 311–363.

GOEZE, J. A. E. 1782 Versuch einer Naturgeschichte der Eingeweidewürmer thierischer Körper. Blankenburg.

GRAHAM, G. L. 1935 *Capillaria* infestations in New Jersey pheasants. J. Parasit. **21**: 61–62.

JOHNSTON, T. H. AND MAWSON, P. 1941 Some nematode parasites of Australian birds. Proc. Linn. Soc. N.S.W. **66**: 3–4, 250–256.

——— AND ———1945 Parasitic nematodes. Rep. British Austr. New Zealand Antarctic Exp. Ser. B **5**: 72–160.

KOTLÁN, A. 1940 Beiträge zur Kenntnis der Parasitenfauna der einheimischen Fasane. Aquila 42–45, 1935–1938, 630–657. (Hungarian, with German summary.)

LOPEZ-NEYRA, C. R. 1947 Los Capillariinae. Mem. Real. Acad. Ciencias Madrid, Ser. Cienc. Natur. **12**: 1–248.

MADSEN, HOLGER 1945 The species of *Capillaria* (Nematodes, Trichinelloidea), parasitic in the digestive tract of Danish gallinaceous game birds, with a revised list of species of *Capillaria* in birds. Danish Review of Game Biology **1**: 1–112.

MOLIN, R. 1858 Prospectus helminthum quae in prodromo faunae helminthologicae venetiae continentur. Sitz. Akad. Wiss. Wien. Math. Nat. Cl. **30**: 127–158.

———1861 Prodromus faunae helminthologicae venetae adjectis disquisionibus anatomicis et criticis. Denkschr. Ak. Wiss. Wien **19**: 189–338.

MOREHOUSE, N. F. 1944 Life cycle of *Capillaria caudinflata,* a nematode parasite of the common fowl. Iowa State Coll. J. Sci. **18:** 217–253.

MORGAN, D. O. 1932 On three species of the genus *Capillaria* from the English domestic fowl. J. Helminth. **10:** 183–194.

OROSZ, D. 1931 *Capillaria* fajok hazi madrainkban (*Capillaria*-Arten des Hausgeflügels). Inaug. Diss. Budapest, 30 pp. (Hungarian, with German summary.)

READ, C. P. 1949 Studies on North American helminths of the genus *Capillaria* Zeder, 1800 (Nematoda). III. Capillarids from the lower digestive tract of North American birds. J. Parasit. **35:** 240–249.

———1950 A new host for *Capillaria caudinflata* (Molin, 1858). J. Parasit. **36:** 91.

REIBISCH, J. 1893 *Trichosomum strumosum* n. sp., ein Parasit aus dem Epithel des Oesophagus von *Phasianus colchicus.* Arch. Naturg. **59:** 331–340.

SCHRANK, F. VON P. 1788 Verzeichnis der bisher hinlänglich bekannten Eingeweidewürmer, München, pp. 1–116.

TODD, A. C. 1946 On the genus *Capillaria* Zeder, 1800, in Tennessee chickens. Trans. Amer. Micr. Soc. **65:** 228–236.

———1947 On *Capillaria bursata* Teixeira de Freitas and Lins de Almeida, 1934. J. Tennessee Acad. Sci. **22:** 191–193.

TUBANGUI, M. A. 1927 Worm parasites of Philippine chickens. Philippine Agric. Rev. **19:** 43 pp.

VAZ, Z. 1939 Redescrição e novos hospedadores de *Capillaria perforans* Kotlán & Orosz, 1931, nematoide patogenico para aves domesticas. Arqu. Inst. Biol. São Paulo **10:** 87–92.

WEHR, E. E. 1936 Earthworms as transmitters of *Capillaria annulata,* the cropworm of the chickens. North Amer. Vet. **17:** 18–20.

———1937 Relative abundance of cropworms in turkeys. Macroscopic differentiation of species. Vet. Medicine **32:** 230–233.

———1939 Studies on the development of the pigeon capillarid, *Capillaria columbae.* U. S. Dept. Agric. Tech. Bull. 679: 19 pp.

YAMAGUTI, S. 1941 Studies on the helminth fauna of Japan, Part 36, Avian nematodes II. Japan J. Zool. **6:** 403–431.

ZUCCHERO, P. J. 1942 Notes on the life cycle of *Capillaria annulata.* Proc. W. Virginia Acad. Sci. **15:** 96–106.

STUDIES ON THE HELMINTH FAUNA OF ALASKA. I. TWO NEW CESTODES FROM SABINE'S GULL (*XEMA SABINI*)

EVERETT L. SCHILLER

Arctic Health Research Center, United States Public Health Service
Anchorage, Alaska

The Territory of Alaska, with its abundant and varied fauna, represents to the biologist one of the few remaining regions of the world where research can be accomplished under conditions of relatively undisturbed ecology. The theoretical interest of the work is greatly enhanced, also by the zoogeographical implications deriving from a region of adjacent continents which have important faunal interrelationships. The distribution and bionomics of the parasitic helminths from hosts occurring in the Arctic-alpine and Hudsonian life zones of Alaska, compared with those from other parts of North America, are of considerable importance. From the zoological standpoint, Alaska is poorly known, and it is particularly evident that the parasitic fauna of the region remains virtually unexplored.

In connection with studies on animal-borne diseases conducted under the direction of Dr. Robert Rausch, by the parasitology branch of the Arctic Health Research Center, a considerable volume of helminthic material is obtained. It is the intention of the staff concerned with these studies to release the resulting information as a series of publications of which the present paper is the first.

Sixteen specimens of Sabine's gull (*Xema sabini*) were collected during the latter part of July and early August of 1949. A single specimen was taken at Point Barrow and the other fifteen at the mouth of the Alaktak River near Admiralty Bay, east of Point Barrow. Of the total number, nine were found parasitized by cestodes representing two species herein described as new.

Haploparaxis xemae, n. sp.
(Plate 1, figs. 1–5)

Diagnosis: Length of strobila about 18 mm.; maximum width at posterior end of strobila 482 μ. Scolex 216 μ in diameter. Suckers muscular about 64 μ in diameter. Evaginated rostellum 256 μ in length and 80 μ in diameter at apex, provided with a single row of 10 hooks, 25.6 μ in length. Strobila 86 μ wide immediately posterior to base of scolex. External segmentation becomes distinct about 1.8 mm. posterior to scolex. Genital *Anlagen* appear early. Genital pores unilateral and dextral, in posterior one-third of proglottid. Cirrus sac slender, averages 266 μ in length by 21 μ in width, extending transversely across median line of proglottid. Seminal vesicle sacculate, 104 μ in length by 52 μ in width in post-mature segments. External seminal vesicle variable in position, ventral to testis, and usually approaching aporal excretory vessel. Globular termination of cirrus heavily spined. Spines about 3 μ in length. Single ovoid testis, very conspicuous throughout most of strobila, occurs aporal to median line and averages 144 μ by 80 μ in mature proglottids. Ovary subspherical, located ventral to cirrus sac. Vitelline gland situated in middle of proglottid between ovary and testis. Vagina lies ventral and posterior to cirrus sac and extends as a slender tube past median line, generally paralleling posterior border of segment, then loops back upon itself and by enlargement forms voluminous seminal receptacle. Seminal receptacle between cirrus sac and ovary, attains a maximum size of 43 μ by 136 μ in post-mature segments. Uterus extends transversely as a slender irregular tube across anterior part of proglottid, contained within boundaries of excretory canals in post-mature segments, becoming sacculate as gravidity is approached, filling entire proglottid when gravid. Ventral longitudinal excretory canals measure 16 μ in diameter; dorsal canals 2 μ in diameter. Average egg size 36 μ by 48 μ. Eggs provided with polar thicknesses. Embryonic hooks 14 μ in length.

Received for publication, September 15, 1950.

Host: Xema sabini (Sabine)
Locality: Alaktak River near Admiralty Bay, Arctic Coast of Alaska.
Habitat: Intestine.
Type: One slide, No. 47346, containing an entire specimen has been deposited in the Helminthological Collection of the U. S. National Museum.

DISCUSSION

As indicated by available host-parasite lists, the parasites of arctic species of gulls have been little studied. Only 3 of the 23 species of cestodes assigned to the genus *Haploparaxis* have been reported from LARIFORMES. Insofar as the writer is aware, *Tetrabothrium cylindraceum* Rudolphi, is the only species of cestode recorded from Sabine's gull. Of the 23 species of *Haploparaxis* known to occur in birds, four are characterized by having a spinose cirrus. Although these four species are found in other than lariform birds, it seems advisable to differentiate them from the species herein described. These were found to differ from *H. xemae* n. sp. as follows:

H. fuliginosa Solowiow, 1911 (parasitic in ANSERIFORMES), although the rostellar hooks have not been observed, differs in having a smaller cirrus sac, which extends less than one-third the distance across the width of the proglottid. It differs, as well, in having eggs devoid of polar thicknesses.

H. scolopacis Yamaguti, 1935 (parasitic in CHARADRIIFORMES) has longer rostellar hooks (30–35 μ) and eggs of larger sizes (54–60 μ in diameter).

H. clerci Yamaguti, 1935 (parasitic in CHARADRIIFORMES) has a strobila of much greater length (130 mm.), a shorter cirrus sac (110–160 μ) which does not reach median line of proglottid and is provided with rostellar hooks of smaller sizes (18–21 μ).

H. veitchi Baylis, 1934 (parasitic in CHARADRIIFORMES) has rostellar hooks of similar length (22–26 μ) but are of a distinctly different shape which is considered to be "atypical" for the genus. The eggs are devoid of polar thicknesses.

New name: Webster (1947) in reviewing the literature regarding the identity of *Taenia fusus* Krabbe, 1869, and the subsequent revision by Fuhrmann (1906 and 1908), in which the name of this species became *Hymenolepis fusus,* has pointed out that the specimens with a single testis described by Joyeux and Baer, 1928, and assigned to *Haploparaxis* (= *Aploparaksis*) *fusus* (= *Taenia fusus* Krabbe, 1869), are by virtue of the first reviser principle, without a name. Since the species decribed by Joyeux and Baer rightfully belongs to the genus *Haploparaxis* and is specifically distinct from the other members of this genus, it is proposed that it be designated *Haploparaxis baeri,* new name.

Key to the genus *Haploparaxis**

1.	Ten rostellar hooks	3
	More or less than 10 rostellar hooks	2
2.	With 46 rostellar hooks	*H. dujardinii*
	With 8 rostellar hooks	*H. australis*
3.	Cirrus spinose	4
	Cirrus aspinose	8
4.	Cirrus pouch extends to less than ⅓ the width of proglottid	5
	Cirrus pouch extends to middle of segment or slightly beyond but does not reach aporal excretory vessel	6

* Hooks of several species of this genus have been figured by Shen Tseng (1932).

5. Cirrus pouch about 73 μ long; eggs 55 μ in diameter; rostellar hooks unknown ..... *H. fuliginosa*
   Cirrus pouch 110–160 μ long; eggs 42–51 × 33–39 μ with polar thicknesses; rostellar hooks 18–21 μ in length ..... *H. clerci*
6. Rostellar hooks 30–35 μ in length ..... *H. scolopacis*
   Rostellar hooks less than 26 μ in length 7
7. Rostellar hook shape "typical" for genus, i.e., having short handle relative to length of guard and blade; hook length 25 μ; eggs with polar thicknesses ..... *H. xemae*
   Rostellar hook shape "atypical" for genus, i.e., having very long handle relative to length of guard and blade; hook length 22–26 μ; eggs without polar thicknesses ..... *H. veitchi*
8. Rostellar hook shape "atypical" for genus 9
   Rostellar hook shape "typical" for genus 10
9. Rostellar hooks 25 μ in length ..... *H. elisae*
   Rostellar hooks 65 μ in length ..... *H. murmanica*
10. Cirrus pouch small, less than ⅓ the width of proglottid 11
    Cirrus pouch extends beyond ⅓ the width of proglottid 15
11. Strobila less than 30 mm. in length 12
    Strobila more than 30 mm. in length 13
12. Strobila 24 mm. long; rostellar hooks 32 μ in length; eggs 34 × 40 μ .. *H. birulai*
    Strobila 6–8 mm. long; rostellar hooks 40 μ in length ..... *H. penetrans*
13. Rostellar hooks less than 33 μ in length 14
    Rostellar hooks 33 μ in length ..... *H. crassirostris*
14. Rostellar hooks 17–19 μ long ..... *H. pseudofilum*
    Rostellar hooks 21–23 μ long; eggs 40 μ in diameter ..... *H. larina*
15. Cirrus pouch extends to middle of segment or slightly beyond but does not reach aporal excretory vessel 16
    Cirrus pouch extends to aporal excretory vessel 21
16. Eggs with two polar thicknesses 17
    Eggs devoid of polar thicknesses 18
17. Rostellar hooks 18.5 μ long ..... *H. filum*
    Rostellar hooks 14 μ long ..... *H. sinensis*
18. Strobila less than 50 mm. in length 19
    Strobila more than 50 mm. in length 20
19. Rostellar hooks 57–60 μ; strobila 45 mm. long; eggs 36 × 45 μ .... *H. japonensis*
    Rostellar hooks 14.3 μ; strobila 20 mm. long; eggs 31 × 47 μ ..... *H. diminuens*
20. Rostellar hooks 18–20 μ; maximum length of strobila 70 mm.; eggs 34 × 36 μ ..... *H. brachyphallos*
    Rostellar hooks 17 μ long; strobila 100–120 mm.; eggs 36 × 50 μ .... *H. baeri*
21. Strobila less than 100 mm. in length 22
    Strobila 150 mm. in length; rostellar hooks 24 μ long ..... *H. cirrosa*
22. Rostellar hooks 48–58 μ long; strobila length 10–35 mm.; eggs 36 μ in diameter ..... *H. furcigera*
    Rostellar hooks 40–44 μ long; strobila length 60 mm. ..... *H. hirsuta*
    Rostellar hooks 20–24 μ long; strobila length 60 mm.; eggs 34 × 40 μ ..... *H. parafilum*

ANSERIFORMES
- *H. birulai* von Linstow, 1905
- *H. elisae* Skrjabin, 1914
- *H. fuliginosa* Solowiow, 1911
- *H. furcigera* (Rudolphi, 1819)
- *H. murmanica* Baylis, 1919
- *H. japonensis* Yamaguti, 1935

CHARADRIIFORMES
- *H. scolopacis* Yamaguti, 1935
- *H. australis* Johnston, 1911
- *H. brachyphallos* (Krabbe, 1869)
- *H. crassirostris* (Krabbe, 1869)
- *H. diminuens* von Linstow, 1905
- *H. clerci* Yamaguti, 1935
- *H. filum* (Goeze, 1782)
- *H. pseudofilum* Clerc, 1902
- *H. hirsuta* (Krabbe, 1882)
- *H. penetrans* Clerc, 1902
- *H. parafilum* Gasowska, 1931
- *H. sinensis* Shen Tseng, 1933

LARIFORMES
- *H. cirrosa* (Krabbe, 1869)
- *H. baeri* new name
- *H. larina* Fuhrmann, 1920
- *H. xemae* n. sp.

PASSERIFORMES
- *H. dujardini* (Krabbe, 1869)

Hymenolepis haldemani, n. sp.
(Plate 2, figs. 1–4)

Diagnosis: Length of strobila about 40–50 mm.; maximum width 1.0 mm. attained at posterior end of strobila. Scolex 160 μ in diameter. Suckers about 64 μ in diameter, unarmed, muscular and directed forward. Invaginated rostellum provided with a single row of 10 hooks, 11.2 μ in length. Strobila 60 μ wide immediately posterior to base of scolex. Genital ducts unilateral and dextral, occurring in anterior one-half of the proglottid, passing dorsal to excretory canals. Muscular cirrus sac averages 250 μ in length by 30 μ in width, extending medially, commonly approaching aporal excretory canal, nearly filling antero-posterior space in mature proglottids. External seminal vesicle pyriform, usually occurring anterior to most distant aporal testis but often loops back ventrally to become situated over aporal end of cirrus sac. Cirrus unarmed and lacking a cirrus stylet. Sacculus accessorius absent. Testes three in number, subspherical to ovoid. Two testes usually occur aporal to ovary and lie in same plane as latter. One testis located poral to ovary in a plane more dorsal to ovary and aporal testes. Ovary lobate. Ovary and vitelline gland located nearly in middle of proglottid. Vagina lies posterior and ventral to cirrus sac. Seminal receptacle not conspicuous. Uterus extends as transverse tube across anterior portion of proglottid and develops by enlargement and lobation, becoming sacculate, filling proglottid when gravid. Ventral longitudinal excretory canals measure 16 μ in diameter; dorsal canals 3 μ in diameter.

Host: Xema sabini (Sabine)

Locality: Alaktak River, near Admiralty Bay, Arctic Coast of Alaska.

Habitat: Intestine.

Type: One slide, No. 47345 containing an entire specimen has been deposited in the Helminthological Collection of the U. S. National Museum.

DISCUSSION

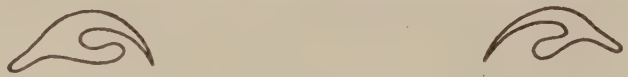
Fourteen of the species of the genus *Hymenolepis* parasitic in aves (*H. parviceps, H. capillaris, H. fusus, H. microcephala, H. kowalewski, H. arcuata, H. fructifera, H. microcirrosa, H. planestici, H. hughesi, H. recurvirostrae, H. recurvirostroides, H. parviuncinata,* and *H. bilharzii*) lack a cirrus stylet, have an aspinose cirrus and possess ten hooks which approach those of *H. haldemani* in size (less than 16 μ in length); however, they all differ markedly in hook shape. The number, size, and characteristic shape of the rostellar hooks together with the conspicuous cirrus sac (nearly filling the antero-posterior space of the mature proglottids) suffice to differentiate the species herein described from all other members of the genus *Hymenolepis*. Additional distinguishing features of lesser importance contribute to further differentiate these forms from *H. haldemani,* but, in view of the more distinct and consistent diagnostic characters discussed above, it is considered unnecessary to enumerate them in detail.

This cestode was named in honor of Dr. Jack C. Haldeman, Medical Officer in Charge, Arctic Health Research Center, Anchorage, Alaska.

Variability: Irregularities in the pattern of testicular arrangement and distribution are not uncommon within proglottids of the same specimen among members of the genus *Hymenolepis*. The writer (1950) reported in some detail on the occurrence of such atypical segments in *Hymenolepis rauschi*. A similar study conducted on the type and paratypes of *H. haldemani* revealed several examples of unusual testicular arrangement worthy of consideration. In some cases the frequency of change in the testes pattern throughout the progression of proglottids made it difficult to determine the "typical" arrangement without first making a critical study of the entire strobila. On the basis of frequency of occurrence of proglottids in which the testes arrangement was similar, the "typical" pattern was found to be of triangular distribution in which two testes are aporal and one poral to the ovary (Plate 2, fig. 4), with the two aporal testes occurring in a plane appreciably more ventral than that of the poral testis. Since in all cases two testes were situated at a plane more ventral than the third, regardless whether in a straight line or a

triangular distribution, the paired testes could be distinguished from the single testis. The most extremely atypical case is considered to be that in which the position is completely reversed with the paired testes occurring on the poral side of the ovary and the single testis aporal to it (Plate 2, fig. 3-b). In the other examples demonstrating variability, the paired testes, as indicated by the depth level, are the two most aporal (Plate 2, figs. 3-a, c, d) and are similar in this respect to the "typical" proglottid.

Synonymy: In connection with this study a large series of hymenolepidid cestodes of LARIFORMES were examined. The literature dealing with these species presents descriptions of two morphologically identical cestodes under different names. Mayhew (1925) described *Hymenolepis fryei* (= *Wardium fryei*), obtained from the intestine of a specimen of *Larus glaucescens,* taken in San Juan County, Washington. In a recent paper, Young (1950) described *Hymenolepis californicus* found in the California gull (*Larus californicus*) and the ring-billed gull (*Larus delawarensis*). In differentiating his specimen from other members of the genus, Young merely stated, "It differs from any species hitherto described in its combination of



Text figure 1. Rostellar hooks of (A) *Hymenolepis fryei* (Mayhew, 1925) and (B) *Hymenolepis californicus* Young, 1950. Rostellar hooks were projected and drawn to the same scale.

characters and it is accordingly described as new." No comparisons enumerating differences between closely related species were given. Since no reference is made to Mayhew's work in 1925, it is assumed that Mayhew's species was overlooked. It is possible that this may have occurred because *Hymenolepis fryei* was originally described under the old generic name of *Wardium.*

Examination of the types of *H. fryei* and *H. californicus* by the writer, was made possible through the kindness of Dr. E. W. Price, Assistant Chief, Zoological Division, Agricultural Research Center, Bureau of Animal Industry. The rostellar hooks from both forms were projected and drawn to the same scale and were found to be identical in size and shape (Text fig. 1). Comparisons of additional characters provide conclusive evidence that these cestodes are morphologically identical and therefore *H. californicus* is considered to be a synonym of *H. fryei* (Mayhew, 1925).

REFERENCES

BAYLIS, H. A. 1934 Some parasitic worms from Australia. Parasitol. **26:** 129–132.

JOYEUX, C. AND BAER, J. G. 1928 Note sur quelques helminthes récoltés en Macédoine. Bull. Soc. Path. Exot. **21:** 214–220.

MAYHEW, R. L. 1925 Studies on the avian species of the cestode family Hymenolepididae. Ill. Biol. Monogr. **10:** 1–125.

SCHILLER, EVERETT L. 1950 *Hymenolepis rauschi* n. sp., a cestode from the ruddy duck. J. Parasit. **36:** 274–277.

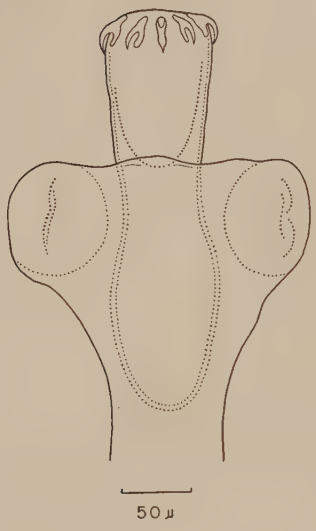
TSENG, SHEN 1932 Studies on avian cestodes from China. Part II. Cestodes from charadriiform birds. Parasitol. **24:** 500–511.

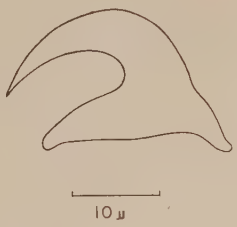
WEBSTER, J. D. 1947 Studies on the genus *Hymenolepis,* with descriptions of three new species. J. Parasit. **33:** 99–106.

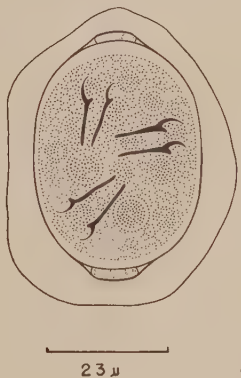
YAMAGUTI, S. 1935 Studies on the helminth fauna of Japan. Part 6. Cestodes of birds, I. Japanese J. Zool. **6:** 183–230.

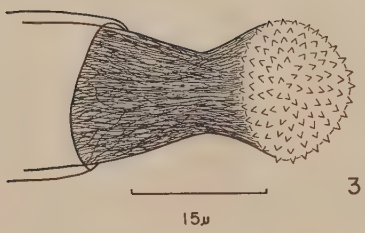
YOUNG, R. T. 1950 Cestodes of California gulls. J. Parasit. **36:** 9–12.

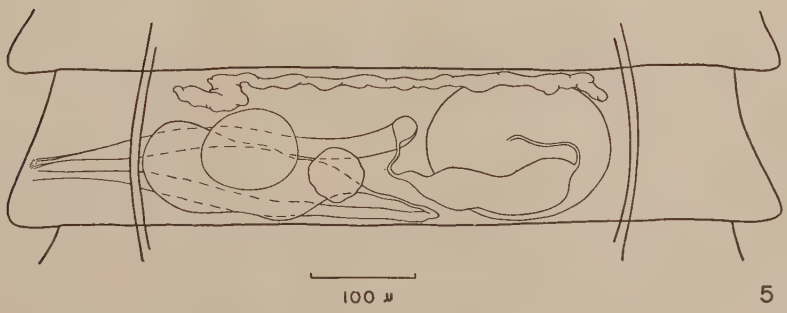
PLATE I









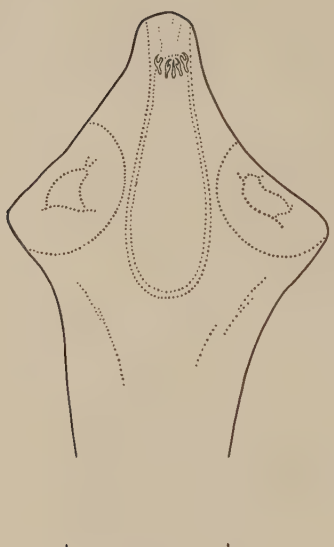


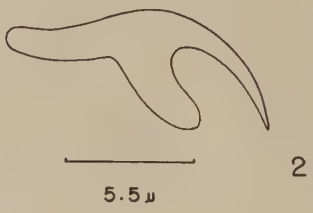
EXPLANATION OF PLATE 1

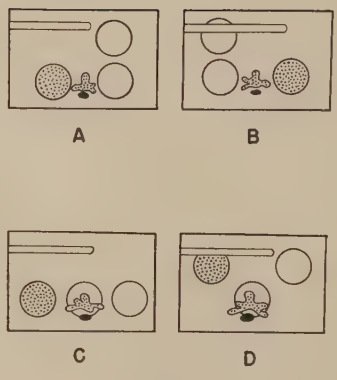
Morphological details of *Haploparaxis xemae* (composite drawing). Figures 1 and 2 drawn with the aid of a projector.

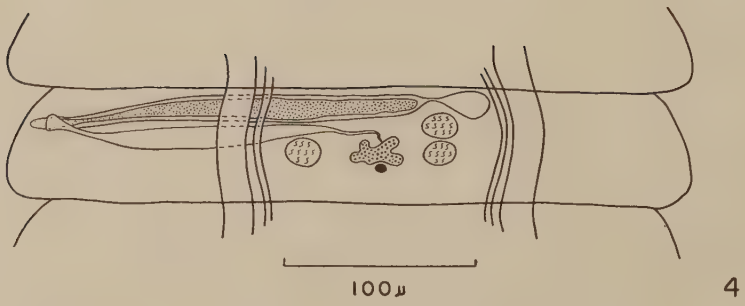
FIG. 1. Scolex.
FIG. 2. Rostellar hook.
FIG. 3. Cirrus.
FIG. 4. Egg.
FIG. 5. Late mature proglottid (ventral view).

PLATE II









Explanation of Plate 2

Morphological details of *Hymenolepis haldemani* (composite drawing). Figures 1 and 2 drawn with the aid of a projector.

Fig. 1. Scolex.

Fig. 2. Rostellar hook.

Fig. 3. Variation in testicular arrangement. Shaded testis most dorsal.

Fig. 4. Early mature proglottid (ventral view).

NOTES ON THE GENUS *GIGANTOLAELAPS* AND DESCRIPTION OF A NEW SPECIES, *GIGANTOLAELAPS CRICETIDARUM* (ACARINA: LAELAPTIDAE)[1]

HARVEY B. MORLAN

Sanitarian (R), Thomasville, Ga.

Fonseca (1939) listed 11 species of his new genus, *Gigantolaelaps*. In the present paper, *Laelaps wolffsohni* Oudemans (1910) and a new North American species are added to the genus.

Fonseca stated that he did not have the necessary bibliography to decide whether or not *Laelaps wolffsohni* Oudemans belonged to *Gigantolaelaps*. From a review of Oudemans' description and figures, it was concluded that *L. wolffsohni* should be transferred to the species of *Gigantolaelaps*.

Laelaps maximus Berlese (1903) was included in *Gigantolaelaps* by Fonseca. Though the original description indicated a similarity to *Gigantolaelaps*, it was inadequate to distinguish Berlese's species from subsequently described members of the genus and therefore has been omitted from the key below.

Fonseca (1939) stated that his *G. mattogrossensis* is similar to *G. peruvianus* (Ewing, 1933), "from which it may be distinguished by the fact that in *G. mattogrossensis* there are two long setae in the basifemur and two in the telofemur, whilst Ewing only reports one seta in each of these joints for his species." Actually, Ewing (1933) stated, "femur and patella of first pair (of legs) each with a seta bearing dorsal protuberance." Cotypes (U. S. National Museum Type No. 1072) of *G. peruvianus* were examined by the author and found to have two longer dorsal setae on femur I (basifemur) and genu I (telofemur or patella); femur II and genu II each with one longer dorsal seta; posterior margin of dorsal plate slightly concave to notched. The description of *G. mattogrossensis* includes characters as follows: posterior margin of sternal plate "slightly convex"; anterior margin of anal plate "slightly convex with central depression"; distal seta of coxa I about 80 μ, proximal spine about 78 μ. In contrast, cotypes of *G. peruvianus* each showed a central concavity in the posterior margin of the sternal plate; anterior margin of anal plate convex, without central depression; distal seta of coxa I 118–132 μ in length, proximal spine about 76 μ.

In describing *G. oudemansi*, Fonseca (1939) observed some specimens had two rather than the customary three small median setae between the anterior pair of long sternal setae. The author examined a specimen (collected by Rockefeller Foundation workers from *Oryzomys ratticeps* at Genipapo, Anapolis, Goiaz, Brazil, on 5–27–46) that fits the description of *G. oudemansi* except that there are 4 small median setae between the anterior pair of long sternal setae.

The following key to the females of *Gigantolaelaps* is, for the most part, based on the literature rather than an examination of actual specimens.

Received for publication, November 6, 1950.

[1] From the Communicable Disease Center, Public Health Service, Federal Security Agency, Atlanta, Ga. Much of the study was completed while the author was a graduate student at Texas Technological College.

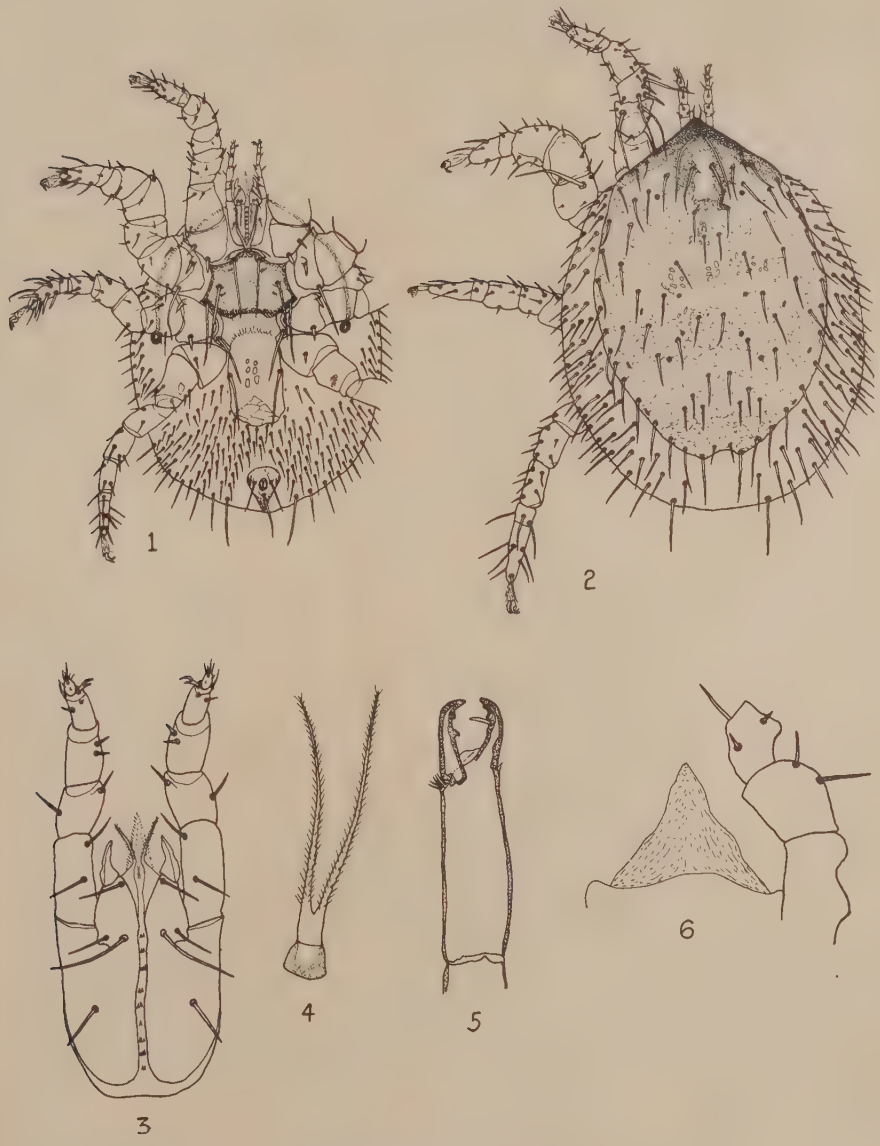
KEY TO THE FEMALES OF *GIGANTOLAELAPS*

1. Sternal plate with 2, 3, or 4 (usually 3) small setae between long anterior pair of setae. *oudemansi* Fons.
   Sternal plate without small setae between long anterior pair of setae 2
2. Coxa I with a single seta ..... *brachyspinosus* (Fons.)
   Coxa I with two setae or spines 3
3. Coxa I with two slender setae, neither spine-like 4
   Coxa I with one or both setae spine-like 8
4. Legs I and II not enlarged, femur of leg I not enlarged ..... *gilmorei* Fons.
   Legs I and II enlarged, femur of leg I enlarged 5
5. Dorsal plate with deeply notched posterior margin ..... *butantanensis* (Fons.)
   Dorsal plate with rounded posterior margin 6
6. Posterior seta of coxa II 2 or 3 times longer than other coxal setae 7
   Posterior setae of coxa II only slightly longer than other coxal setae ..... *versteegi* (Oud.)
7. Base of tritosternum covered by sternal plate ..... *wolffsohni* (Oud.)
   Base of tritosternum not covered by sternal plate ..... *comatus* Fons.
8. Coxa I with both setae spine-like 9
   Coxa I with proximal seta spine-like, distal seta slender, not spine-like 10
9. Dorsal plate with rounded posterior margin, sternal plate 360–400 μ in length. *goyanensis* Fons.
   Dorsal plate with notched posterior margin, sternal plate 310–350 μ in length. *cricetidarum* n. sp.
10. Dorsal plate with rounded posterior margin, anterior margin of sternal plate reaches or partially covers base of tritosternum ..... *vitzthumi* Fons.
    Dorsal plate with slightly concave to notched posterior margin, anterior margin of sternal plate does not reach base of tritosternum 11
11. Posterior margin of sternal plate convex, distal seta of coxa I only slightly longer than proximal spine ..... *mattogrossensis* (Fons.)
    Posterior margin of sternal plate with central concavity, distal seta of coxa I over half again as long as proximal spine ..... *peruvianus* (Ewing)

Gigantolaelaps cricetidarum n. sp.

Female: Figs. 1–6. Similar to but smaller than *Gigantolaelaps goyanensis* Fonseca, 1939. Sternal plate broader than long, heavily sclerotized, with 3 pairs of long gradually pointed setae and the usual 2 pairs of slit-like pores; more lightly sclerotized in area between insertion of anterior pair of setae; sharp lateral angles extend between coxae I and II and coxae II and III; thick margins are concave laterally, convex posteriorly often with two submedian posterior projections, anteriorly strongly projected to cover base of tritosternum. Tritosternum pilose from point of bifurcation, reaching to base of pedipalp trochanter. Genitoventral plate reticulated, posteriorly slightly expanded and with a larger more pronounced network of lines; 3 pairs of lighter subcircular markings grouped near center of plate and slightly anterior to insertion of the single pair of setae. Endopodal plate narrow, extending from sternal plate to middle of coxa IV, more sclerotized at the insertion of its seta between coxa III and IV. Roughly triangular metapodal plate at level of trochanter IV, two very small plates on each side of the genitoventral plate. Anal plate triangular, reticulated, with darker rounded anterior corners; paired setae, at level of anal pore, shorter than the unpaired seta. Peritreme extends from opening of stigma, between coxa III and IV, forward to the area between coxa I and II where it turns to follow the margin of the dorsal plate to its anterior extremity. Stigma without posterior prolongation. Unsclerotized venter striate with about 90 pairs of setae which are longer along the posterior margin. Dorsal plate reticulated, with especially heavy sclerotization along the anterior margin and centrally extending irregularly back from the pointed anterior extremity of the plate to enclose a longitudinally directed oblong area of lighter sclerotization; numerous elliptical markings in center of plate; 4 pairs of round and 3 pairs of slit-like pores; about 40 pairs of setae, posterior margin of plate notched. Uncovered tergum striate with about 54 pairs of setae which are longer at the posterior margin. Leg I noticeably larger than III and IV, but not as large as leg II. Leg IV longest. Coxa I with two spine-like setae, proximal heavier than distal. Coxa II with an anterior spine-like seta and a posterior long seta. Coxa III with two spine-like setae. Coxa IV with single seta which is shorter and weaker than setae of other coxae. Femur I with two longer dorsal setae, genu I with two longer dorsal setae of which the proximal is longest. Dorsal surface of femur II and genu II each with a single longer seta. Spine-like setae on tarsi II, III, and IV, wider in tarsus III. Tectum (epistome), seen only in dissected specimens, membranous with broad base tapering to a point slightly beyond level of pedipalp

PLATE I



EXPLANATION OF PLATE I

Gigantolaelaps cricetidarum n. sp.

FIG. 1. Ventral view of female.
FIG. 2. Dorsal view of female.
FIG. 3. Ventral view of gnathosoma of female.
FIG. 4. Tritosternum of female.
FIG. 5. Chelä of female.
FIG. 6. Tectum (epistome) and base of pedipalp of female.

trochanter. Hypostome with 9 rows of 1 to 3 teeth per row. Larger, movable arm of chelae with 2 teeth below the curved apex and a semicircle of setae at its base; immovable arm with 2 teeth and slender pilus dentilus below notched, curved extremity; minute, inflated seta at base. The average and range of measurements in microns of 10 to 12 specimens are:

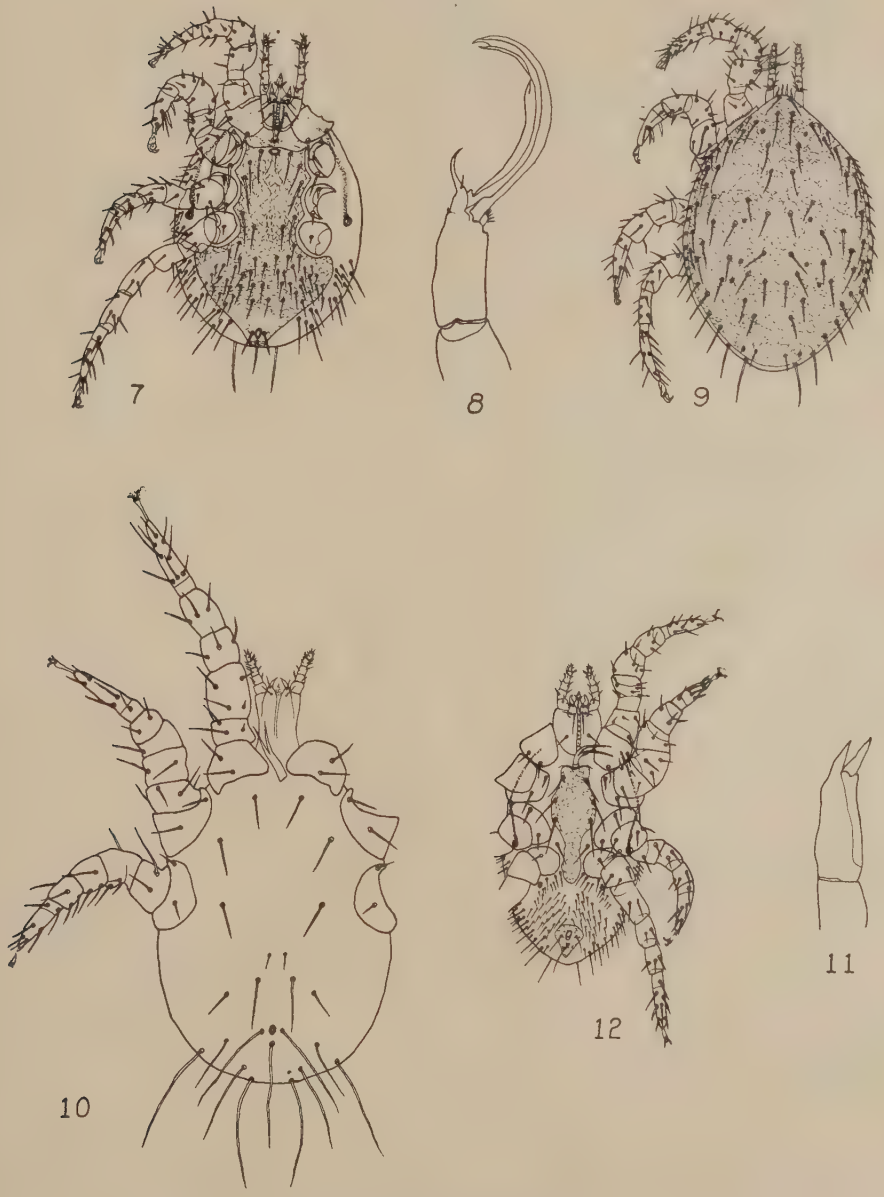
Total length to apex of palps	1850	1749–1978
Total length, exclusive of gnathosoma	1592	1457–1714
Width of body at widest point	1166	985–1388
Length of dorsal plate	1478	1444–1492
Width of dorsal plate	979	902–1034
Length of sternal plate at mid-ventral line	328	312– 347
Width of sternal plate at narrowest point	376	347– 402
Width of sternal plate at widest point	443	402– 472
Anterior seta of sternal plate	288	278– 312
Median seta of sternal plate	277	271– 333
Posterior seta of sternal plate	317	291– 333
Endopodal seta	290	250– 326
Length of genitoventral plate from posterior margin to base of sternal plate	566	527– 604
Width of genitoventral plate at widest point	324	270– 368
Width of genitoventral plate at level of genital seta	276	208– 291
Length of genital seta	256	229– 271
Length of anterior projection of sternal plate	110	104– 118
Space between genitoventral and anal plates	206	83– 326
Width of anal plate at widest point	211	194– 271
Length of unpaired anal seta	202	166– 243
Length of paired anal seta	153	146– 166
Space between anterior margin of anal plate and anterior margin of anal pore	40	28– 48
Metapodal plate	81	69– 104
Outer posterior submedian seta of dorsal plate	214	194– 222
Inner posterior submedian seta of dorsal plate	76	62– 83
Length of proximal spine of coxa I.	77	69– 83
Longest dorsal seta of femur I (basifemur)	370	347– 402
Long dorsal seta of femur I (basifemur)	339	278– 375
Longest dorsal seta of genu I (telofemur)	300	291– 312
Long dorsal seta of genu I (telofemur)	242	208– 264
Length of posterior seta of coxa II	316	278– 340
Long dorsal seta of femur II (basifemur)	323	298– 347
Long dorsal seta of genu II (telofemur)	141	118– 153

Male: Figs. 7–9. Idiosoma 1110 μ in length by 715 μ in width at level of leg IV, lightly sclerotized. Holoventral plate with nearly straight anterior margin, laterally projecting between coxae, greatly expanded below coxae IV, reticulation coarser in anterior portion than in expanded area, 3 pairs of sternal setae progressively longer from anterior to posterior, endopodal setae between coxae III and IV, genital pair of setae at level of coxae IV, unpaired anal seta longer than paired anal setae, about 23 pairs of setae in the expanded area of plate, 3 pairs of lighter oblong marks near center of plate at level of coxae IV. Uncovered venter striate, with 30–35 pairs of setae. Stigma between coxae III and IV, peritreme visible to middle of coxa I. Dorsum almost entirely covered by lightly sclerotized, reticulated plate with 35–40 pairs of setae. Legs of same proportions as female, coxal setae of same arrangement but shorter and finer than in female, spine-like seta on femur, tibia, and tarsus of leg II. Movable arm of chelae with semicircle of setae at base, grooved spermatophore carrier sickle-shaped; immovable arm relatively short and narrow, a fine seta inserted proximal to curved portion, minute inflated seta at base of arm.

Although similar to *Gigantolaelaps goyanensis,* the male of the new species is smaller, expanded area of holoventral plate with fewer setae and with 3 pairs of lighter markings on plate near genital setae, lacking the deeply colored anal zone and the very strong spines in trochanter and genu of leg II.

Deutonymph: Fig. 12. Similar to but less sclerotized than female; idiosoma (of the single specimen) 1215 μ in length by 763 μ in greatest width at level of leg III; sternal plate reticulated with narrow extension posteriorly continued to slightly below base of coxae IV, with only 3 pairs of long sternal setae. Metapodal plate below trochanter IV. Anal plate like female but without darker areas in anterio-lateral corners. Peritreme visible to coxa I. Tritosternum similar to female. Uncovered venter striate with 40–45 pairs of setae. Legs proportioned like female, coxal setae like female but weaker. Tarsus I with fine setae, tarsi II, III, and IV with

PLATE II



EXPLANATION OF PLATE II

Gigantolaelaps cricetidarum n. sp.

FIG. 7. Ventral view of male.
FIG. 8. Chela of male.
FIG. 9. Dorsal view of male.
FIG. 10. Ventral view of larva.
FIG. 11. Chela of larva.
FIG. 12. Ventral view of deutonymph.

some broader setae; femur I dorsally with two longer setae. Dorsal plate reticulated, lightly sclerotized, covering almost entire dorsum, setation similar to female. Gnathosoma like female.

Larva: Figs. 10–11. Whitish in color, idiosoma averaged (in the two specimens) 989 μ in length by 767 μ in width at leg III. Venter without apparent sclerotization, all plates lacking, 3 pairs of long setae with insertions near base of coxae I, II, and III, very long paired setae at level of anal pore with odd seta of at least equal length below anal pore, 7 other pairs of seta on venter. Stigma and peritreme lacking. First pair of legs longer than other two, leg II heaviest; two setae on each coxae. Dorsum without plates, with 14 pairs of setae. Chelae without apparent teeth or setae.

Holotype: Female (ex TM-50, *Oryzomys palustris palustris,* Thomas County, Georgia, December 7, 1946, H. B. Morlan, collector) deposited in the U. S. National Museum, No. 1930.

Allotype: Male (ex *Oryzomys palustris,* Savannah, Georgia, Wildlife Refuge, April 18, 1946, C. M. Tarzwell, collector) deposited in the U. S. National Museum.

Paratypes: Three females and a larva mounted on one slide (On *Oryzomys palustris,* Bull's Island, South Carolina, March 25, 1939, W. P. Baldwin, collector, Bishopp N. 29614, Lot 40-7123) deposited in the U. S. National Museum together with 2 slides containing a deutonymph and a female with the same collection data given for the allotype.

Other paratypes deposited at the Rocky Mountain Laboratory, Hamilton, Montana (same collection data as holotype), and Communicable Disease Center Museum, Atlanta, Georgia (same collection data as allotype).

Records. During an extensive survey for murine typhus in southwestern Georgia animals (Morlan, Hill, and Schubert, 1950), the new mite was found from only two hosts. In addition to the 46 females from 14 rice rats, *Oryzomys palustris palustris* (Harlan), previously reported by Morlan and Strandtmann (1949), 3 female mites were taken from 1 of 966 cotton rats, *Sigmodon hispidus komareki* (Say and Ord). Collections made by C. M. Tarzwell at the Savannah, Georgia, Wildlife Refuge included 41 females, 2 males, and 1 deutonymph from 7 rice rats and 1 cotton rat. Specimens received from the U. S. National Museum included 3 females and a larva collected by W. P. Baldwin, March 25, 1939, at Bull's Island, South Carolina, on *Oryzomys palustris;* 1 female collected by C. N. Smith, January 25, 1944, Bull's Island, South Carolina, on rice rat; 1 female collected by E. W. Jameson, Jr. at Port Arthur, Texas, April 9, 1944, from *Oryzomys palustris.* Two females were received from D. B. Lieux, who reports (personal communication) the collection of 2 females each from *Rattus rattus alexandrinus* and *Rattus norvegicus* on September 29, 1948, at Miami, Florida. Worth (1950) records that 35 per cent of 40 rice rats, *Oryzomys palustris coloratus* Bangs, taken in the Everglades near Homestead, Florida, during January 1949 harbored *Gigantolaelaps* sp.

Remarks. Eggs, larvae, and in one case a protonymph were found on dissection of female specimens, indicating that birth may be given to the first nymphal stage. Unfortunately the single protonymph was too badly damaged to permit description. The new mite has been named for the family (Cricetidae) of mammals which includes the principal host, *Oryzomys palustris.*

SUMMARY

Gigantolaelaps cricetidarum n. sp. is described from rodents, primarily *Oryzomys palustris,* collected in Georgia, Florida, South Carolina, and Texas. *Laelaps wolffsohni* Oudemans is transferred to *Gigantolaelaps.* Notes, additional to the original descriptions of *Gigantolaelaps peruvianus* (Ewing) and *Gigantolaelaps oudemansi* Fonseca, and a key to the females of 13 species of *Gigantolaelaps* are given.

ACKNOWLEDGMENTS

Thanks are due and grateful acknowledgment is made to Dr. R. W. Strandtmann, Professor of Biology at Texas Technological College, for advice, criticisms, and encouragement; Dr. J. C. Cross, Chairman, Department of Biology at Texas Technological College, for encouragement and for making facilities available for use; Dr. E. W. Baker for making specimens from the U. S. National Museum available for study; Dr. H. D. Pratt and Dr. C. M. Tarzwell for specimens from the Communicable Disease Center Museum; and Mr. E. V. Komarek, Thomasville, Georgia, for aid and advice in the collection and determination of mammals.

References

Berlese, A. 1903 Description of *Laelaps maximus.* Redia **1**: 259.

Ewing, H. E. 1933 New genera and species of parasitic mites of the superfamily Parasitoidea. Proc. U. S. Natl. Museum **82**: 1–14.

Fonseca, Flavio da 1939 Acarological Notes XXV. The giant Laelaptidae, parasites of South American rodents; new genus and species (Acari). Mem. Instit. Butantan **12**: 57–102.

Morlan, Harvey B., Hill, Elmer L. and Schubert, Joseph H. 1950 Serological survey for murine typhus infection in southwest Georgia animals. Pub. Health Rep. **65**: 57–63.

Morlan, Harvey B. and Strandtmann, R. W. 1949 The occurrence of neotropical mites in the United States. J. Parasit. **35**: 217.

Oudemans, A. C. 1904 *Laelaps versteegi* nov. sp. Entomol. Berichten **1**: 160.

———— 1904 *Laelaps versteegii,* a new species of parasitic mite. Notes Leyden Mus. **24**: 223–231.

———— 1910 Nouvelle espèce d'Acarien du Chili. Rev. Chilena Hist. Nat. **14**: 147–151.

Worth, C. Brooke 1950 Observations on ectoparasites of some small mammals in Everglades National Park and Hillsborough County Florida. J. Parasit. **36**: 326–335.

BLOOD PARASITES FROM CALIFORNIA DUCKS AND GEESE

CARLTON M. HERMAN[1]

Bureau of Game Conservation, California Division of Fish and Game

During the period 1941 through 1949 blood smears were procured from 1,011 wild ducks and geese obtained from various sources in California. The birds studied were either suffering from botulism or fowl cholera, trapped for migration studies, shot by hunters, or collected specifically for obtaining blood samples. Samples were procured from the peripheral blood in the living birds and from the heart in dead birds. The author is indebted to the several persons who aided in obtaining the samples reported in this paper.

Blood smears were stained with Giemsa's stain and examined with the aid of the oil immersion objective of a compound microscope for at least ten minutes.

Findings are summarized in Table 1. Not included in the table are the following, all of which were negative: four Canada geese (*Branta canadensis canadensis*), four cackling geese (*Branta canadensis minima*), three white-fronted geese (*Anser albifrons*), three snow geese (*Chen hyperborea*), thirty gadwalls (*Chaulelasmus streperus*), two wood ducks (*Aix sponsa*), nine canvasbacks (*Nyroca valisineria*), fifteen redheads (*Nyroca americana*), one ring-necked duck (*Nyroca collaris*), one greater scaup (*Nyroca marila*), four lesser scaup (*Nyroca affinis*), seven ruddy ducks (*Erismatura jamaicensis*), and two mergansers (*Mergus merganser*).

TABLE 1.—*Incidence of Blood Parasites in California Ducks and Geese*

	Number Examined	Number Infected	Plasmodium	Leucocytozoon	Haemoproteus	Microfilaria
Mallard	368	2	..	1	1	1
Cinnamon Teal	71	1	1	..	..	..
Green-winged Teal	126	7	..	..	3	4
Pintail	263	7	5	2	..	..
Baldpate	43	2	..	2	..	1
Shoveller	55	9	..	6	3	..

Haemoproteus hermani was observed in the mallard (*Anas platyrhynchos*), green-winged teal (*Anas carolinensis*), and shoveller (*Spatula clypeata*). These were all morphologically similar to the *Haemoproteus* previously reported from North American ducks (Herman, 1938; Nelson and Gashweiler, 1941; Wetmore, 1941; Wood and Herman, 1943) and from the black duck in Egypt (Haiba, 1948). However, the host records for this parasite from the shoveller and green-winged teal are new.

All the *Leucocytozoon* observed in this series were diagnosed as *L. simondi*. Host records include mallard, pintail (*Dafila acuta tzitzihoa*), baldpate (*Mareca americana*) and shoveller. This parasite has been reported as an important pathogen of ducks in some areas, particularly in the Mississippi Flyway of North America (O'Roke, 1934) where a high incidence was reported in ducklings. The infrequency of occurrence of this parasite in the ducks reported in the present series, particularly its absence in a large series of ducklings from Honey Lake, Lassen

Received for publication, November 20, 1950.

[1] Now of the U.S. Fish and Wildlife Service, Patuxent Research Refuge, Laurel, Maryland.

County, might indicate that *Leucocytozoon* is not the problem in the Pacific Flyway that it is in the central portion of North America.

Microfilaria were seen only in smears obtained from heart blood from the mallard, green-winged teal and baldpate. No adult filaria was observed at autopsy of any of these ducks.

The most significant finding in this series of blood slides was the occurrence of *Plasmodium* spp. in several ducks. Although in recent years domestic ducks have been used extensively as experimental hosts in malaria studies there have been no reports of natural infection with *Plasmodium* in native North American species of ducks. There have been a few reports of *Plasmodium* in captive ducks at zoological parks in other parts of the world. Schaudinn (1911) and Scott (1928) reported *P. praecox* from the swan (*Cygnus melanocoryphus*) and Plimmer (1915) recorded the same parasite from a falcated duck (*Eunetta falcata*). Gilruth, Sweet and Dodd (1910) described a new species, *P. biziurae*, from two captive musk ducks (*Biziura lobata*) in Australia and Cleland (1915) reported this parasite from a black swan (*Chenopsis atrata*). The morphology of the parasite described by Gilruth *et al* is very similar to *P. relictum*, having round, dot-like pigment granules in characteristic round gametocytes which displace the host-cell nucleus. Unfortunately their material contained no asexual forms and therefore they were unable to describe the segmenting stages.

In the present study, similar plasmodia were observed in an adult male pintail from Tulare Lake in September, 1942, an adult male pintail from Mt. Eden, Alameda County on January 15, 1944, a four weeks old pintail and a three weeks old cinnamon teal (*Querquedula cyanoptera*) from Honey Lake during July, 1943. In all but the pintail duckling only mature gametocytes or young developing forms were observed. In the pintail duckling a number of developing segmenters were seen and an apparently mature schizont contained 14 merozoites. The parasites have round, dot-like pigment and displace the nucleus of the host cell. The gametocytes are more or less round, sometimes filling most of the available area in the host-cell cytoplasm and sometimes occupying a central area roughly 2/3 that of the former but equally displacing the host-cell nucleus. It is believed that the plasmodial infections in all four of these ducks was the same and should be classified as *Plasmodium relictum* (= *P. biziurae*).

Another *Plasmodium* was observed in two immature pintails from Gridley during December, 1948. Few parasites were seen. Elongate gametocytes were observed which differed from *Haemoproteus hermani* by their more irregular outline and tendency to occupy only a portion of the cytoplasm of the host cell on one side of the nucleus. Several developing segmenters were seen, one with 12 recognizable merozoites. Too few parasites were observed to permit a specific diagnosis and these infections are reported simply as *Plasmodium* sp.

Blood smears were made from 16 mallards about 2 weeks old procured from Lake Merritt in the city of Oakland, May 2, 1949. Blood smears were obtained from 10 of these ducklings one week later and from three of these after another week. All remained negative.

Blood from five of the wild ducklings captured at Lower Klamath Refuge in July, 1949 was inoculated into five domestic Pekin ducks (four weeks old). Blood smears were examined every other day for 40 days with negative results.

SUMMARY

Blood smears were procured from 1,011 geese and ducks of 19 species from various locations in California. Parasites were found in 28 individuals. The parasites observed included *Haemoproteus hermani, Leucocytozoon simondi,* microfilaria, *Plasmodium relictum* (= *P. biziurae*), and *Plasmodium* sp. with elongate gametocytes. This is the first report of a natural infection with a *Plasmodium* in North American wild ducks.

REFERENCES

CLELAND, J. B. 1915 The haematozoa of Australian birds. No. 3. Trans. Proc. Roy. Soc. S. Aust. **39**: 25–37.

GILRUTH, J. A., SWEET, G. AND DODD, S. 1910 Notes on blood parasites. Proc. Roy. Soc. Victoria **23**: 231–241.

HAIBA, M. H. 1948 Plasmodia of common Egyptian birds. J. Comp. Path. **58**: 81–93.

HERMAN, C. M. 1938 *Haemoproteus* sp. from the common black duck, *Anas rubripes tristis.* J. Parasit. **24**: 53–56.

NELSON, E. C. AND GASHWEILER, J. S. 1941 Blood parasites of some Maine waterfowl. J. Wildl. Mngt., **5**: 199–205.

O'ROKE, E. C. 1934 A malaria-like disease of ducks. Univ. Michigan Sch. Forest and Cons. Bull., **4**: 44 pp.

PLIMMER, H. G. 1915 Report on the deaths which occurred in the Zoological Gardens during 1914. Proc. Zool. Soc. London, 123–130.

SCHAUDINN, F. 1911 In Prowazek, Handbuch der Pathogen Protozoen.

SCOTT, H. H. 1928 Report on the deaths occurring in the Society's Gardens during the year 1927. Proc. Zool. Soc. London, 81–119.

WETMORE, P. W. 1941 Blood parasites of birds of the District of Columbia and Patuxent Research Refuge vicinity. J. Parasit. **27**: 379–393.

WOOD, S. F. AND HERMAN, C. M. 1943 The occurrence of blood parasites in birds from southwestern United States. J. Parasit. **29**: 187–196.

GAMETOGENESIS, FERTILIZATION AND CLEAVAGE IN THE TREMATODE, *ZYGOCOTYLE LUNATA* (PARAMPHISTOMIDAE)

CHARLES H. WILLEY AND GABRIEL C. GODMAN

New York University, University College of Arts and Science

The germ cell cycle has been studied more or less completely for a considerable number of species of digenetic trematodes. Britt (1947) described the number and behavior of the chromosomes in species representing 8 families, and reviewed reports in the literature on chromosome numbers in 10 other families. The contributions of White (1940), Jones (1945) and Britt (1947) were primarily concerned with gathering cytological evidence for an evolutionary mechanism in the PLATYHELMINTHES.

The present paper is concerned with a cytological investigation of the germ cells in the adult *Zygocotyle lunata,* of the family PARAMPHISTOMIDAE. Spermatogenesis, oögenesis, fertilization, early cleavage and chromosome morphology are described. Britt (1947) included in his tabulation of studies on chromosome numbers in TREMATODA the work of Cary (1909), in which the amphistome *Diplodiscus temperatus* is described as having a diploid number of 16 chromosomes. However, Cort (1915) showed that Cary in his life history studies was not dealing with *Diplodiscus,* but had described two different species of larval trematodes neither of which belonged to *Diplodiscus temperatus.* Neither was an amphistome cercaria, and Cort figured and described them from Cary's material as *Cercaria caryi* and *Cercaria megalura.* Therefore the reports on the chromosome number of *Diplodiscus temperatus* are not valid, and except for an abstract (Willey and Godman, 1941) on gametogenesis in *Zygocotyle,* no information is hitherto available in the literature on the chromosomes and the nuclear phenomena of the germ cell cycle for any of the PARAMPHISTOMIDAE.

The complete life cycle of *Zygocotyle lunata* was described by Willey (1937, 1938, 1941). Ample material consisting of specimens of a wide variety of known ages was available for cytological study. Preliminary studies dealing mostly with spermatogenesis in this species were performed in 1941 and presented as a thesis by the junior author. In a published abstract of this work, (Willey and Godman, 1941) the diploid chromosome number of *Z. lunata* was reported as 14. Subsequent work by the senior author has substantiated and extended the earlier observations to include more information on oögenesis, fertilization and cleavage.

A graded series of 25 specimens obtained from the caeca of experimentally infected hosts (rats, ducks) and varying in age from 23 days (immature) to 394 days was fixed in either mercuric chloride with 3% acetic acid or in Flemming's strong, chrom-osmic-acetic solution. The worms were imbedded in paraffin and serially sectioned at 5 microns. Twelve of the specimens were stained in Heidenhain's iron-alum haematoxylin with a light-green counterstain. The others were treated according to a modified Weigert iron-haematoxylin technique (Lee, 1937) in which ferric chloride is mixed directly with an alcoholic solution of haematoxylin. This process leaves the chromosomes bluish and was found to be fairly satisfactory. There are very considerable differences to be noted with regard to the effects of

Received for publication, December 20, 1950.

the fixatives employed and the staining reactions. The corrosive sublimate-acetic mixture brings out the chromosomes well but cytoplasm and achromatic structures are poorly differentiated. Flemming-fixed material presents a finely granular cytoplasm and shows spindle fibers and centrioles which are invisible with the corrosive sublimate fixation. In addition the chromosomes are well differentiated. All drawings were made from sections and some with the aid of a camera lucida. Photomicrographs were obtained using Bausch and Lomb Type R photomicrographic equipment with tungsten arc and Eastman Kodak M plates. Illustrations are not all made to the same scale but relative sizes are indicated in the text.

OBSERVATIONS

The Chromosomes. The number and morphology of the chromosomes in species of digenetic trematodes are important in elucidating the evolutionary mechanism as well as taxonomic relationships in the PLATYLHELMINTHES. The chromosome number has been reported for many species but in some of these forms the studies were conducted mainly on the meiotic chromosomes which are short and heavily pycnotic and hence afford little information on the number of chromosome arms or the positions of the centromeres. As stated by White (1945), "It is much more useful to know the number of chromosome arms, the extent and position of heterochromatic regions, the location of nucleolar organizers, the chiasma frequencies of the arms, and so on." Until such information is available for a large number of species in a group, any conclusion concerning its chromosomal evolution would be only tentative. Since satisfactory observations on chromosome morphology are obtainable from cells of somatic tissues, sections of specimens of *Zygocotyle* of various ages were searched for cells undergoing division. In both immature and mature specimens of all ages, dividing cells were found frequently among those of the intestinal caeca and the subcuticular tissues. They showed 14 chromosomes, the form of which is identical with that of the chromosomes of cells in early cleavage stages.

The first and second cleavages proved to be the most favorable material for study of chromosome morphology. As seen in the first cleavage (Figs. 31, 47) there are two pairs of acrocentric chromosomes about 5.5 μ long; two pairs between 4.0 and 4.5 μ long, one of which is acrocentric and the other metacentric; two pairs of acrocentrics between 3.0 and 3.5 μ long; and one pair of small metacentric chromosomes about 3.0 μ in length. Figure 36 represents a single genom for *Zygocotyle lunata*. Chromosomes identified in dividing intestinal and subcuticular cells and in primary spermatogonia, while the same in number and form, were shorter by about 0.5 μ to 1.0 μ than those seen in early cleavage stages. The chromosomes exhibit their characteristic form in all cells dividing mitotically, but it has not been possible to identify the individual meiotic chromosomes in the first maturational division with particular members of the genom (Fig. 36).

Spermatogenesis. The testes vary in appearance and staining capacity with the degree of sexual maturity of the specimen. In the immature worm, the testicular wall is not pronounced and the cells of the testis are closely compacted. Spermatogonia predominate and are arranged in several close but poorly defined layers along the wall of the testis. In mature worms, the wall of the testis is a thick fibrous sheath continuous around the organ. The testicular wall is not so well demon-

strated in specimens fixed in corrosive sublimate-acetic, but in preparations made with Flemming's fluid the inner contents of the organ tend to shrink away from the wall, leaving it exposed. Primordial germ cells lie within the fibrous sheath toward its inner surface (Fig. 1). Germ cells show a more or less distinct orientation in the testis. Primordial germ cells and spermatogonia are peripherally located, while spermatocytes and spermatids are more central in position. According to Severinghaus (1927) and Niyamasena (1940) this condition does not exist in the schistosomes. In *Zygocotyle* spermatogenesis precedes oögenesis. In a 23-day immature specimen spermatids are found in the testes, while the ovary is still only a cord of primordial germ cells. Anderson (1935), Chen (1937) and others have described masses of small cells in the testes of trematodes, which they suggested might be nutritive material. Cable (1931), Rees (1939) and Willey & Koulish (1950) reported no such degenerating spermatogonia or any other nutritive cells in the testes of *Cryptocotyle, Parorchis* and *Gorgoderina,* respectively. No degenerating cells or material which could be interpreted as nutritive could be found in the testes of *Zygocotyle.*

The primordial germ cells which lie within or adhere closely to the fibrous tunica of the testis are especially numerous in immature specimens. They are ovoid and measure about 4.5 microns. The nucleus contains two homogeneous nucleoli and the chromatin is clumped into irregular, deeply staining masses. They undergo multiplicative division and figure 1 shows the prophase of a mitotic division in which 14 chromosomes can be counted. The primordial cell undergoes a growth period to become a mature primary spermatogonium. Intermediate cells in the growth process are between 5 and 6 μ in diameter and tend to protrude from the inner surface of the testicular wall, while full-grown primary spermatogonia maintain a connection with the wall at only one point, measuring at this stage 8 μ in diameter. The cytoplasm and cell boundary is distinct and the resting nucleus, containing 2 nucleoli and dispersed, finely divided chromatin, is eccentrically located (Fig. 2). Further increase in size to a diameter of 10 μ is followed by division and in the prophase (Fig. 3) of dividing primary spermatogonia, the chromosomes stain heavily and are well differentiated. The 7 pairs present the same form and size as is found in somatic cells of the worms. Each daughter cell (secondary spermatogonium) receives 14 chromosomes since ordinary mitosis occurs.

At the conclusion of the first spermatogonial division, the secondary spermatogonia remain together (Fig. 4). Each is hemispherical, the straight sides being mutually contiguous. A growth period ensues at the end of which each cell is spherical and in contact with its neighbor at one point. Each secondary spermatogonium then divides mitotically. The plane of this division is perpendicular to that of the first, resulting in a cluster of 4 cells, the tertiary spermatogonia (Fig. 5). They appear as 4 nuclei closely invested with a common cytoplasmic sheath. In each of the interphase nuclei, two prominant nucleoli appear. Division of each of the 4 tertiary spermatogonia produces a rosette of 8 cells which are the primary spermatocytes. They undoubtedly possess the diploid number of chromosomes before their prophase. When first formed, they measure about 4.5 μ and the nucleus lies in the free rounded end of each cell.

The prophase of the primary spermatocyte involves growth and chromosomal activities leading to the meiotic divisions. In the earliest stage the chromatin forms

delicate, thread-like leptotene strands (Fig. 7). In the zygonema the threads shorten and take the stain more heavily. Syndesis (synapsis) presumably occurs at this time. The zygotene threads are best seen in preparations fixed with corrosive sublimate-acetic fluid. In Flemming-fixed material synezesis is always apparent (Fig. 8). In this case the chromatin contracts into an intensely basiphilic mass. The next stage is the pachynema in which a number of thickened loops appears (Fig. 9). These loops are all oriented with their open or free ends toward the center of the cluster. Examination of favorable pachytene loops indicates a tendency to doubling. It was difficult to make counts at this stage because of crossing of loops and aberrations caused by sectioning, but 7 loops have been tentatively traced. Pseudo-reduction has occurred through pairing of homologous chromosomes. In the diakinesis which follows the pachytene, the chromosomes become progressively shorter to form the 7 tetrads.

At the conclusion of the prophase, the primary spermatocytes measure about 8 μ and as the metaphase of the first meiotic division begins, 7 tetrads (bivalents) appear on the spindle. They are thick, deeply-staining bodies which show characteristic rods, rings and cruciform shapes. Figure 10a shows the division of primary spermatocytes. Centrioles are clearly evident. Chen (1937) reported that in *Paragonimus* the centrioles are patent through most of the stages, dividing at each pole in every division up to that of the secondary spermatocyte, where they remain single. In *Zygocotyle* the spindle is clearly visible only in the first meiotic division, and then only in Flemming's-fixed material. Similarly, Rees (1939) observed that in *Parorchis* the bipolar spindle of the first maturation division is the only spindle visible in spermatogenesis.

Following the first meiotic division, each daughter cell is one of a cluster of 16 secondary spermatocytes formed by the simultaneous division of the 8 primary spermatocytes. Each measures 5 μ and all are centrally connected. They are com-

Explanation of the Plates

Plate I

Spermatogenesis in *Zygocotyle lunata,* Figs. 1–17.

Fig. 1. Prophase of a dividing primordial germ cell in the testis, showing 14 chromosomes.

Fig. 2. Mature primary spermatogonium, showing its relation to the testicular wall.

Fig. 3. Prophases of division of 3 primary spermatogonia.

Fig. 4. Anaphase of dividing primary spermatogonium.

Fig. 5. A group of tertiary spermatogonia.

Fig. 6. Anaphase of division of a cluster of tertiary spermatogonia followed over 2 sections.

Fig. 7. Section through a rosette of primary spermatocytes, showing 5 of the 8 cells in the leptonema stage.

Fig. 8. Section through group of primary spermatocytes in the zygonema, showing synezesis. Flemming fixation.

Fig. 9. Section of a cluster of primary spermatocytes showing 6 of the 8 cells in the pachynema.

Fig. 10a and 10b. Two sections of a group of dividing primary spermatocytes in metaphase, showing tetrads and spindles.

Fig. 11. Section of a group of primary spermatocytes in late anaphase.

Fig. 12. Section showing 11 of the 16 cells of a rosette of secondary spermatocytes.

Fig. 13. Section through a cluster of dividing secondary spermatocytes.

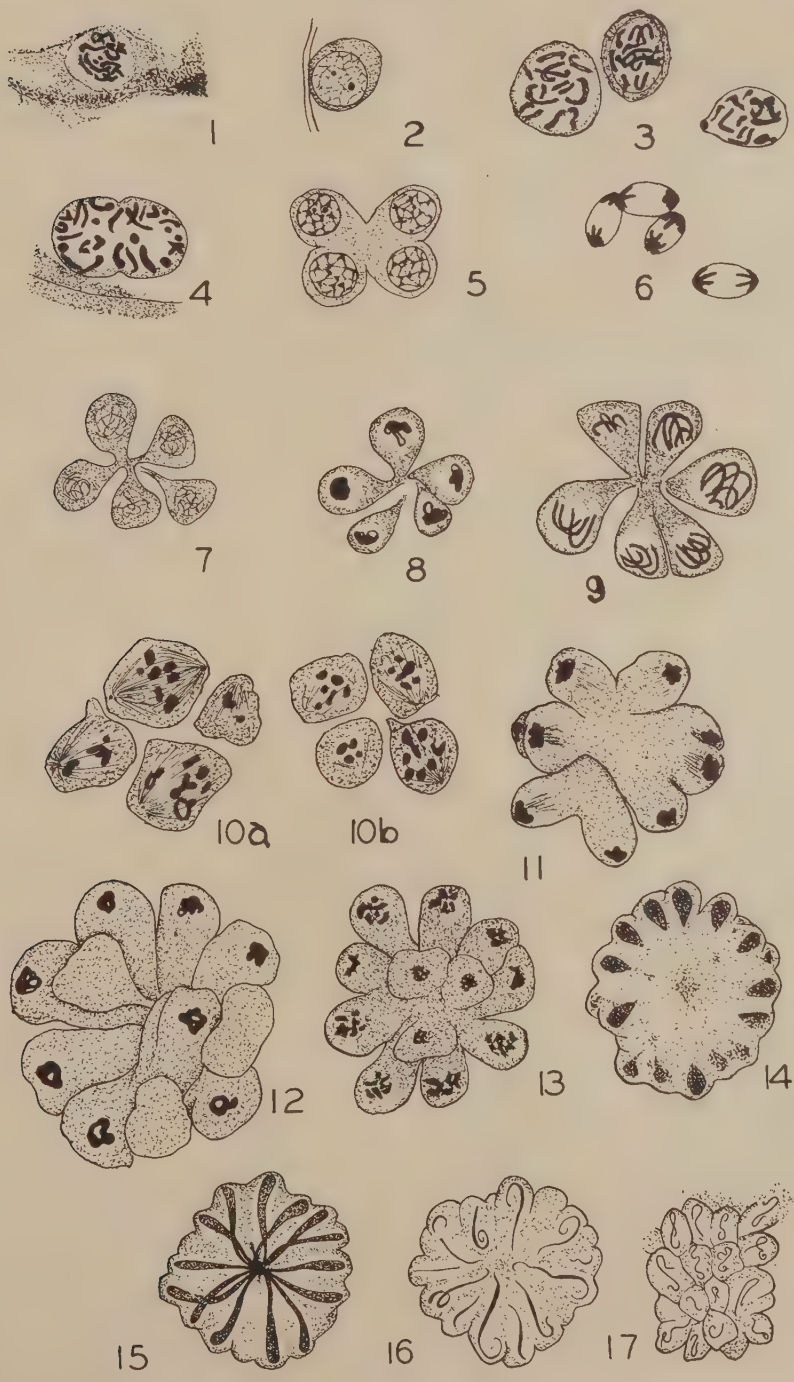
Fig. 14. Section through a spermatid cluster showing the first stage of nuclear condensation and elongation.

Fig. 15. Section of spermatid group showing later stage in nuclear elongation.

Fig. 16. Section of spermatid group showing late nuclear elongation.

Fig. 17. Section of spermatid group showing coiled sperm threads.

PLATE I



monly seen as very faintly stained cells whose nuclei tend to be oxyphilic rather than basiphilic. However, before the subsequent division of the secondary spermatocyte a nuclear reorganization occurs in which the chromatin condenses and becomes intensely basiphilic. These condensation nuclei are characterized by ring-figures and clumps (Fig. 12). In the ensuing division, the chromosomes appear in sections of prophase and metaphase stages in the form of short rods or large granules (Fig. 13). The haploid number, 7, is clearly evident. No observation could be made as to which maturational division is reductional in the true sense, since the homologous chromosomes are not heteromorphic.

The second meiotic division produces a cell cluster of 32 spermatids. The cell outlines, which were fairly distinct around the secondary spermatocytes, can be detected only as indentations at the free surface of the group of spermatids which now lie in a common cytoplasmic mass (Fig. 14). The first nuclear change in the spermatid involves a condensation of the chromatin which is at first intensely basiphilic. The nucleus becomes ovoid with the narrow end oriented toward the center of the spermatid cluster. The chromatin assumes a granular appearance (Fig. 15) and shows less affinity for basic dyes. Further elongation is accompanied by a loss of the granular appearance and an increased basiphilic tendency, until the nuclei ultimately become threadlike (Fig. 17) and stain intensely. These threads then uncoil within the cytoplasm and the spermatozoa emerge in long, sheaf-like bundles, leaving behind a residual body of cytoplasm which has a diameter of about 20 μ. The extended spermatozoan measures about 50 μ in length (Fig. 37). A slightly heavier head part is about 10 μ long and the sperm body tapers into a long delicate tail, with no visible differentiation.

Oögenesis, Fertilization and Cleavage. The ovary of a 23-day old specimen (immature) consists of a cord of solidly packed primordial germ cells and young

PLATE II

Oögenesis and cleavage in *Zygocotyle lunata*. Figs. 18–36.

FIG. 18. A resting oögonium in the ovarian sheath.

FIG. 19. An oögonial anaphase.

FIG. 20. A young oöcyte with 2 smaller oögonia.

FIG. 21. Leptonema of the meiotic prophase of the oöcyte.

FIG. 22. Zygonema of the meiotic prophase of the oöcyte.

FIG. 23. Pachynema of the meiotic prophase of the oöcyte.

FIG. 24. Stage of oöcyte prophase, interpreted as the diplonema, which immediately precedes the diffuse stage.

FIG. 25. A full-grown, resting oöcyte showing the characteristic eccentric nucleolus and the condition of the cytoplasm and its components. Cf. Fig. 38.

FIG. 26. Oöcyte in first meiotic division, showing tetrads. Sperm lies in cytoplasm. From sections of cell seen in Fig. 39.

FIG. 27. Oöcyte in second meiotic division, showing formation of second polocyte adjoining the first and an early stage of sperm condensation. From sections of cell seen in Figs. 40, 41.

FIG. 28. Mature ovum, female pronucleus in advanced stage and male pronucleus just forming. From sections of cell seen in Figs. 42, 43.

FIG. 29. Ovum showing male and female pronuclei of equal size (12 μ) at beginning of resting stage. From sections of cell seen in Figs. 44, 45.

FIG. 30. Later stage showing resting pronuclei reduced to 7 μ in diameter. Centriolar apparatus is clearly evident. From sectioned cell seen in Fig. 46.

FIG. 31. Metaphase of first cleavage in transverse section. Drawn from sections of cell seen in Fig. 47.

FIG. 32. Section of late anaphase of first cleavage. Cf. Fig. 48.

FIGS. 33, 34, 35. Early cleavage showing 3, 4 and 5-cell stages as seen in Figs. 49–52.

FIG. 36. Single genom of *Zygocotyle lunata*.

PLATE II

oögonia. In older specimens a definite fibrous ovarian wall immediately surrounds the oögonia and oöcytes are located more centrally within the ovary. Oögonia are best studied in immature specimens. They are irregularly shaped cells measuring about 4.5 μ. The nucleus of each contains a single large nucleolus (Fig. 18). They undergo multiplicative division and small but poorly defined mitotic figures appear in most sections (Fig. 19).

Young, inactive oöcytes, found scattered near the center of the ovary, are rounded and average about 6.0 μ in diameter. At the beginning of the extended prophase, which is coincident with a period of growth, a network of delicate leptotene threads appears (Fig. 21). This network condenses into coarse loops, at which time synapsis presumably occurs. The pachytene loops, which stain faintly with iron-haematoxylin, are oriented with their free ends toward the pole of the nucleolus (Fig. 23). The loops transform into 7 intensely staining chromosomes which appear doubled and obviously represent the diplonema (Fig. 24). Immediately following this stage the oöcyte enters the diffuse condition, wherein the chromatin becomes finely divided and during which much growth occurs. At the outset of the diffuse stage the oöcyte measures about 7.5 μ. The full-grown primary oöcyte (Figs. 25, 38) averages between 18 and 20 μ; it is somewhat elongated and has a nucleus about 10 μ in diameter. A large, conspicuous nucleolus lies characteristically in contact with the nuclear membrane. The chromatin is so finely divided and evenly distributed that the oöcyte nuclei appear almost hyaline. The cytoplasm contains many spherical bodies of varying sizes which blacken with osmic acid and are concerned with nutrition.

The question of the mode of nutrition of trematode oöcytes has received various interpretations in the literature. Katheriner (1904) and Goldschmidt (1908) found yolk nuclei in the oöcyte cytoplasm of *Gyrodactylus elegans* and *Dicrocoelium lanceatum* respectively. Gille (1914) confirmed Katheriner's observations on *Gyrodactylus* and declared that the yolk nuclei are aborted oöcytes which nourish the developing oöcyte. Gille further noted that the abortive egg cells are taken in at the pole of the growing oöcyte where the eccentric nucleolus is located. He described pseudopodia-like extensions from the nucleus at the same pole that the abortive oöcytes are taken in. Both Cable (1931) and Chen (1937) expressed the

---

PLATE III

Photomicrographs showing oögenesis and cleavage in *Zygocotyle lunata*.

FIGS. 37–52. All from sections except FIG. 52.

FIG. 37. Anterior end of spermatozoan.

FIG. 38. Resting oöcyte as depicted in Fig. 25.

FIG. 39. Oöcyte in first meiotic division, closely applied to egg shell. Cf. Fig. 26.

FIGS. 40, 41. Successive sections of oöcyte in second meiotic division, depicted in Fig. 27.

FIGS. 42, 43. Two different focal planes of mature ovum showing advanced stage of female pronucleus and early stage of male pronucleus, as drawn in Fig. 28.

FIGS. 44, 45. Two different focal planes of ovum containing large male and female pronuclei as illustrated in Fig. 29.

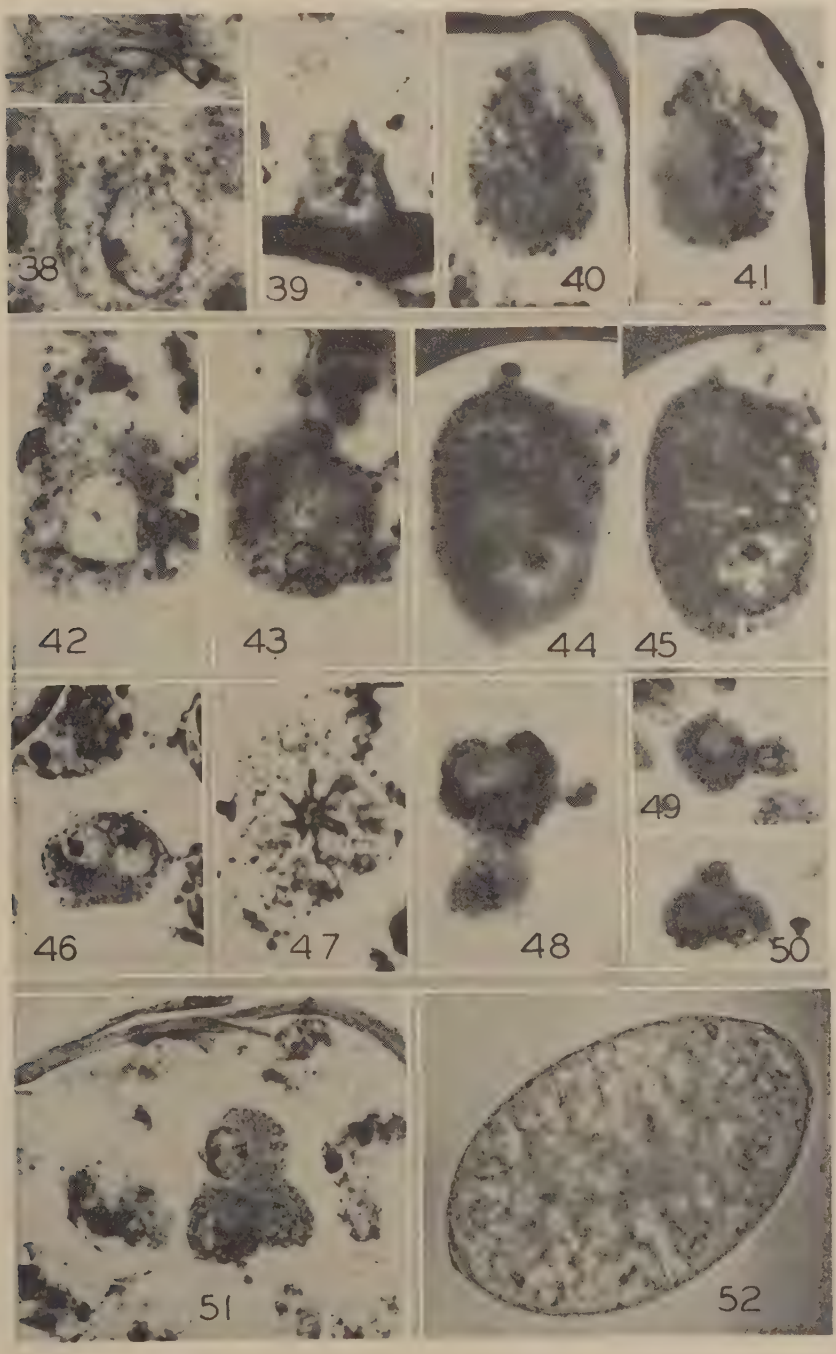
FIG. 46. Ovum in resting stage, with small (7 μ) pronuclei. Cf. Fig. 30.

FIG. 47. Single focal plane of cell drawn in Fig. 31, showing metaphase of first cleavage.

FIGS. 48–51. Sectional views of early cleavage stages.

FIG. 52. Egg, living. Length 0.145 mm. The embryo, showing unequal blastomeres, appears between the center and the opercular end (at right). Eggs are voided at this stage with the feces of the host.

PLATE III



belief that the germ cells are nourished while in the gonads by the body fluid, and not by the degeneration of cells of the germinal line.

The cytoplasm of the oöcyte of *Zygocotyle lunata* contains rounded bodies which may be yolk granules but no degenerating cells that might be regarded as nutritive are to be seen. It is therefore to be inferred that nourishment for the growing oöcytes is derived from the body fluid which permeates the intercellular spaces. Willey & Koulish (1950) found suspended reserve food masses interpreted as nutritive material in oöcytes in the ovary of *Gorgoderina attenuata,* and stated that, "No degenerating cells or nuclei which might act as food for the oöcyte were observed in the ovary of *Gorgoderina attenuata.* Furthermore, these reserve food masses in the oöcytes stain negatively with the Feulgen method, providing evidence against the belief that they are engulfed yolk nuclei as reported in earlier studies (Goldschmidt 1905), and against the belief of Anderson (1935) and others that they are the pyknotic nuclei of degenerating oöcytes absorbed by normal oöcytes."

The full-grown primary oöcytes (Figs. 25, 38) with nuclei in the diffuse stage pass from the ovary into the oviduct and proceed to the oötype region. Fertilization occurs in the oviduct by the entrance of a single, entire spermatozoan. The oöcyte is thus activated and the two maturational divisions follow. In the oötype regions the oöcyte is surrounded by vitelline cells released in groups from the vitellaria, and soon a shell membrane appears enclosing the vitelline cells and the oöcyte to form the egg, which passes through the uterus where maturation, formation of male and female pronuclei, and early cleavage divisions ensue.

The meiotic phenomena of the maturing ovum have not been observed in digenetic trematodes as completely as those of spermatogenesis. Many of the published reports on gametogenesis either omit entirely or refer only briefly to the progress of maturation of the ovum within its shell. This is largely due to the difficulty of sectioning the eggs, the brittle shells of which shatter and tear the tissues during cutting, and to the shrinkage of the contents of the egg because of poor penetration through the shell during fixation. Examination of numerous mature specimens of *Zygocotyle* has afforded a fairly complete sequence of these stages.

Immediately after penetration of the spermatozoan, the primary oöcyte, surrounded by yolk cells and enclosed within a thick shell, resumes its meiotic activities. The chromatin condenses to reveal 7 deeply staining tetrads and the nucleolus and nuclear membrane disappear (Figs. 26, 39). The chromosomes at this stage exhibit the same shapes and sizes as they do in the dividing primary spermatocyte. The sperm lies off to one side of the achromatic figure and begins to shorten. The first meiotic division forms the first polar body and the secondary oöcyte. In all specimens of *Zygocotyle* observed, the oöcytes engaged in either of the maturational divisions lay at or near the periphery of the egg in close proximity to the shell, and the polar bodies were produced from the side of the oöcytes nearest to the shell. Figures 39, 40, 41, 44 and 45 are photomicrographs of sections showing the orientation of the oöcytes with respect to the heavily blackened shell. In the second meiotic division (Figs. 27, 40, 41) the secondary oöcyte divides to form the second polar body and the ovum. In *Zygocotyle,* the second polocyte is cut off close to the first. The achromatic figure is large but not well developed and no centrioles or asters were observed in either of the divisions. The chromosomes appearing during the second division are not as short and compact as those of the heterotypic (first) divi-

sion and can to some extent be identified individually with those seen in cleavage stages and in somatic cells undergoing mitosis. The second polocyte, appearing as a large bulge at first (Fig. 27) and containing a haploid set of chromosomes, becomes smaller and leaves most or all of the cytoplasm behind in the ovum before it pinches off completely from the surface. When fully formed and still attached to the cell membrane, the first polar body measures about 3.3 μ and the second is about 4.2 μ in diameter. The chromosomes of the polocytes break down into chains of numerous small chromatic granules, which later appear to swell so that the polar bodies are seen as small opaque spheres, which remain attached to the ovum during early cleavage.

While the meiotic divisions are taking place the sperm continues to shorten and condense (Figs. 27, 41) in preparation for its ultimate association with the female pronucleus. The maturation divisions occur rapidly and are always found in the first part of the uterus, behind the ovary and slightly anterior to the acetabulum. The cytoplasm presents a homogeneous appearance with relatively fine granules, and this contrasts strongly with the coarse granulation of the surrounding yolk cells. The size of the oöcyte changes very little during maturation. It averages 19 μ in diameter and its nucleus is from 8 to 10 μ in size. It is not possible to tell which of the meiotic divisions is reductional. Pseudoreduction has occurred by the synapsis of the homologues, but whether they segregate in the first or in the second meiotic division is entirely unknown for *Zygocotyle* or for any other species of the TREMATODA.

After both polocytes are formed the nuclear membrane of the ovum reappears and the nucleus recedes to the center of the cell and becomes vesicular. Its chromatin appears as thin radiating strands of finely divided material which stains very lightly, and the nucleolus reappears. The ovum at this stage averages from 20 to 25 μ and its nucleus (the female pronucleus) is about 12 μ in diameter (Figs. 28, 42). The sperm, still small as compared with the female pronucleus (Figs. 28, 42, 43), now enlarges rapidly and becomes vesicular. The chromatin loosens and a network of delicate strands appears homogeneously distributed through the nuclear framework as the gamete becomes the male pronucleus. A double centriole appears between the pronuclei while the male pronucleus is still small. It persists as the forerunner of the centriolar apparatus of the first cleavage. Each of the pronuclei enlarges to about 12 μ in diameter (Figs. 29, 44, 45) and then decreases to 7 μ in the fertilized ovum which is 20 μ in diameter at this time (Figs. 30, 46). A large and conspicuous nucleolus is present in each of the pronuclei which are in intimate contact. This stage is encountered in the sections with greater frequency than any of the preceding stages and it is inferred that the ovum remains in this resting stage for a considerable time before the cleavage process sets in. Search through the adjoining sections of a resting ovum usually reveals the presence of the double centrioles with astral rays destined to contribute to the formation of the first cleavage amphiaster. This stage is found in the uterus about midway between the ovary and posterior testis.

Eggs in the uterus at a level just behind the posterior testis contain ova showing pronuclear union and first cleavage. Chromatin in the pronuclei increases in staining capacity as the nucleoli disappear and seven chromosomes emerge from each as the nuclear membrane breaks down. A typical amphiaster develops and the

chromosomes pass into the equatorial plane where they intermingle and divide, and the first cleavage ensues (Figs. 31, 47). This cleavage is unequal and produces a large cell which varies in size from 17 to 19 μ (Average 17.5 μ) and a small one varying from 8 to 11 μ (Average 9 μ). The next cleavage involves only the larger cell, giving rise to a 3-cell stage. The large cell, as measured in 18 eggs, averaged 14 μ in size after the second cleavage and the 2 smaller cells, of about equal size are 9 μ in diameter. The large blastomere remains in a resting state through at least the following two cleavages in which only small blastomeres take part. At the 5-cell stage the large cell is still 14 μ and the smaller ones are each about 7 μ in diameter. These measurements are from fixed tissues and would be larger in living eggs. Three-cell stages are found in the uterus at about the level of the posterior testis and embryos of 4 and 5 cells appear in eggs near or anterior to the anterior testis. None were found beyond the 5-cell stage and apparently the embryos do not ordinarily develop further until after they emerge from the genital pore and are passed out into the water with the feces of the host. Figure 52 is a photomicrograph of a living embryonated egg, 0.145 mm. long, collected from the feces of a duck. The single large blastomere and one of the smaller blastomeres of the embryo appear a short distance from the opercular end of the egg.

DISCUSSION

Since *Zygocotyle lunata* is the only species of the PARAMPHISTOMIDAE for which the chromosomes and their behavior are known, conclusions based on cytological evidence, relative to the evolutionary mechanism involved in the origin of the amphistomes and to the taxonomic relationships within the family, must await information on the chromosomes of other species of the group. The processes of gametogenesis, fertilization and cleavage are not significantly different from what has been reported for other digenetic trematodes.

Identification of the meiotic division at which true reduction (segregation) occurs is not possible for any of the monoecious digenetic trematodes, because maternal and paternal chromosomes are indistinguishable in this group where heteromorphic pairs are as yet unknown. As pointed out by Willey & Koulish (1947, 1950) the first maturational division of certain trematodes has been described by some authors as reductional. No evidence is available to support such a conclusion. Following synapsis to form bivalents, true reduction occurs during the meiotic divisions which follow, in either the heterotypic (first) or homeotypic (second) division.

Britt (1947) and Ciordia (1949, 1950) in cytological investigations of chromosome behavior in trematodes sectioned their material at 12 μ. Ciordia (1949) stated, "Sections were cut at 12 micra, since thinner sections may be cytologically untrustworthy." Ordinarily such thick sections interfere with precise observation of finer cytological detail. It is true that thick sections may favor the interpretation of long chromosomes but in describing the chromosomes of *Rhopalias macracanthus* Ciordia stated, "The shortest chromosomes were about one micron long and the longest were 2.4 micra long." The advantage of studying such material from sections 12 μ in thickness is not apparent. Perhaps with thinner sections it would have been possible to identify more precisely the cells from which observations were recorded.

In a paper on the life history of *Z. lunata*, Willey (1941) stated that, "The ovum, measuring from 20 μ to 25 μ in diameter, still unsegmented, lies embedded between the vitelline masses in the opercular half of the egg." Observations were limited at that time to living material and only after examination of eggs in sectioned material was it discovered that the ovum usually undergoes cleavage to the 5-cell stage by the time the egg leaves the genital pore. In none of the hundreds of eggs observed in the anterior part of the uterus in sections of the adult, had the ovum failed to cleave.

Bennett (1936) in a study of the life history of the amphistome *Cotylophoron cotylophorum*, reported that, "Eggs are usually deposited before cleavage begins, but occasionally they are deposited as far advanced in cleavage as the four-cell stage." Variation in the stage of development in *C. cotylophorum* may be due to the method used in collecting the eggs. Bennett stated, "The eggs used in making these observations were secured by taking adult worms from the host and placing them in dishes of water where they would deposit eggs for several hours." In studies on the life history of the amphistome *Diplodiscus temperatus*, Krull and Price (1932) found that if worms were taken from the host and placed in water they would deposit, over a period of time, eggs containing embryos in varying degrees of development. The first eggs deposited were in a more advanced stage of development of the miracidium than those deposited later. The variation in degree of development of eggs obtained by this method may be explained as the more rapid extrusion of eggs into the water than would occur normally within the host, where they would pass through the uterus more slowly and hence be laid in a more advanced stage. Actually, little significance is to be attached to the question of whether such eggs as those of *Z. lunata* or *C. cotylophorum* are deposited before cleavage or in the 4- or 5-cell stage, since from two to three or more weeks in water are required for development to the stage at which the miracidium hatches from the egg. The process is more rapid at the higher summer temperatures.

As a result of preliminary studies (Godman and Willey, 1941) on further development of the embryo in the eggs of *Z lunata*, it was tentatively concluded that this species shows an early segregation of a germinal "propagatory" or "stem" cell as described in *Paragonimus kellicotti* by Chen (1937) and in *Fasciolopsis buski* by Ishii (1934). However, subsequent attempts to confirm and extend the observations have been inconclusive and the evidence in support of such an early differentiation in the TREMATODA is as yet not convincing.

SUMMARY

Morphology of the chromosomes is described and the diploid number found to be 14 in somatic tissue cells, early blastomeres, spermatogonia and oögonia. Behavior of the chromosomes throughout spermatogenesis and oögenesis is followed from specimens varying in age from 23 days (immature) to 394 days. Study of the relation between the cytoplasmic constituents of oöcytes and the nutrition of the ovum leads to the conclusion that the developing ovum receives in its cytoplasm no abortive or degenerating oöcytes from the ovary to contribute to its nutrition. Fertilization in the oviduct activates the oöcytes to undergo the usual 2 meiotic divisions, after which a resting female pronucleus is formed contiguous with a male resting pronucleus which becomes organized rapidly after the polocytes are cut off.

These stages and the ensuing early cleavages occur at definite and constant levels in the uterus of the worm.

Literature Cited

Anderson, M. G. 1935 Gametogenesis in the primary generation of a digenetic trematode, *Proterometra macrostoma* Horsfall, 1933. Trans. Amer. Micr. Soc. **54**: 271–297.

Bennett, H. J. 1936 The life history of *Cotylophoron cotylophorum*, a trematode from ruminants. Ill. Biol. Monogr. **14** (4): 1–119.

Britt, H. G. 1947 Chromosomes of digenetic trematodes. Amer. Nat. **81**: 276–296.

Cable, R. M. 1931 Studies on the germ-cell cycle of *Cryptocotyle lingua* Creplin. I. Gametogenesis in the adult. Quart. J. Micr. Sci. **74**: 563–590.

Cary, L. R. 1909 The life history of *Diplodiscus temperatus* Stafford. With especial reference to the development of the parthenogenetic eggs. Zool. Jahrb., Abt. Anat. Ont. **28**: 595–659.

Chen, Pin-Dji 1937 The germ cell cycle in the trematode, *Paragonimus kellicotti* Ward. Trans. Amer. Micr. Soc. **56**: 208–236.

Ciordia, Honorico 1949 Cytological study of *Rhopalias macracanthus* Chandler, 1932, a trematode from the opossum, *Didelphis virginiana*. J. Parasit. **35**: 417–422.

———1950 The chromosomes of *Notocotylus filamentis* Barker, 1915, a monostome from the muskrat (*Fiber zibethicus*). Trans. Amer. Micr. Soc. **69**: 64–65.

Cort, W. W. 1915 Some North American larval trematodes. Ill. Biol. Monogr. **1**: 449–532.

Gille, K. 1914 Untersuchungen über die Eireifung, Befruchtung und Zellteilung, von *Gyrodactylus elegans* von Nordmann. Arch. Zellfors. **12**: 415–456.

Godman, G. C. and Willey, C. H. 1941 Notes on embryonating eggs of *Zygocotyle lunata* and on their preparation for cytological study. J. Parasit. **27** (suppl.): 15.

Goldschmidt, R. 1908 Über das Verhalten des Chromatins bei der Eireifung und Befruchtung des *Dicrocoelium lanceatum* Stil. et Hass. (*Distomum lanceolatum*). Arch. Zellfors. **1**: 232–244.

Ishii, Yoshio 1934 Studies on the development of *Fasciolopsis buski*. Part 1. Development of the eggs outside the host. J. Med. Assoc. Formosa **33**: 349–379.

Jones, A. W. 1945 Studies in cestode cytology. J. Parasit. **31**: 213–235.

Katheriner, L. 1904 Über die Entwicklung von *Gyrodactylus elegans* von Nordmann. Zool. Jahrb., Festschr. Weissmann, Suppl. **7**: 519–550.

Krull, W. H. and Price, Helen F. 1932 Studies on the life history of *Diplodiscus temperatus* Stafford from the frog. Occas. Papers Mus. Zool., Univ. Mich. No. **237**: 1–41.

Lee, A. Bolles 1937 The microtomist's vade-mecum. 10th Ed. Blakiston, Philadelphia.

Niyamasena, S. G. 1940 Chromosomen und Geschlecht bei *Bilharzia mansoni*. Zeits. Parasit. **11**: 691–701.

Rees, G. 1939 Studies on the germ cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part 1. Anatomy of the genitalia and gametogenesis in the adult. Parasitol. **31**: 417–433.

Severinghaus, A. E. 1927 Sex studies on *Schistosoma japonicum*. Quart. J. Micr. Sci. **71**: 653–702.

White, M. J. D. 1940 Evidence for polyploidy in the hermaphrodite groups of animals. Nature (London) **146**: 132–133.

———1945 Animal cytology and evolution. Cambridge Univ. Press, London, 1–375.

Willey, C. H. 1937 The development of *Zygocotyle* from *Cercaria poconensis* Willey, 1930. Jour. Parasit. **23** (suppl.): 571.

———1938 The life history of *Zygocotyle lunata*. Jour. Parasit. **24** (suppl): 30.

———1941 The life history and bionomics of the trematode, *Zygocotyle lunata* (Paramphistomidae). Zoologica, N. Y. Zool. Soc. **26**: 65–92.

——— and Godman, G. C. 1941 Gametogenesis in the trematode, *Zygocotyle lunata*. Anat. Rec. **81** (suppl.): 78.

——— and Koulish, S. 1947 Gametogenesis in *Gorgoderina attenuata*. Anat. Rec. **99** (suppl.): 640.

——— and ———1950 Development of germ cells in the adult stage of the digenetic trematode, *Gorgoderina attenuata* Stafford, 1902. J. Parasit. **36**: 67–79.

PYGIDIOPSOIDES SPINDALIS N. GEN., N. SP., (HETEROPHYIDAE; TREMATODA), AND ITS SECOND INTERMEDIATE HOST

W. E. Martin

Zoology Department and the Hancock Foundation
University of Southern California

Naturally infected brackish-water fishes, *Fundulus parvipinnis parvipinnis* (Girard), were collected at Newport Bay, California and fed to cats and to newly-hatched chicks. After a lapse of four to six days the cats and chicks were found to harbor small trematodes in the small intestine which do not fit any of the existing genera although they obviously are heterophyids.

MATERIALS AND METHODS

Living specimens and material fixed in Bouin's solution and stained with paracarmine were studied.

OBSERVATIONS

Pygidiopsoides n. gen.

Diagnosis: Small, oval to spindle-shaped trematodes. Mouth surrounded by a single row of large spines. Oral sucker oval in lateral view not tapering posteriorly into a cone. Prepharynx, pharynx, and esophagus well-developed, ceca terminating near posterior border of ventral sucker. Ventral sucker near mid-body level, well-developed, and enclosed in a ventro-genital sac. Single testis in posterior one-third of body. Large seminal vesicle bipartite; "prostate" well-developed; cirrus and cirrus sac lacking. Ovary between testis and acetabulum; Laurer's canal present; uterus in mature specimens filling most of posterior half of body; eggs oval, operculate, relatively large; vitellaria transverse between testis and ovary. Large, muscular, common genital ejector present. Ventro-genital sac contains two, lateral, lenticular gonotyls. Excretory bladder simple, spindle-shaped to tubular; excretory pattern of the "mesostoma" type. Remnants of eyespots may be present.

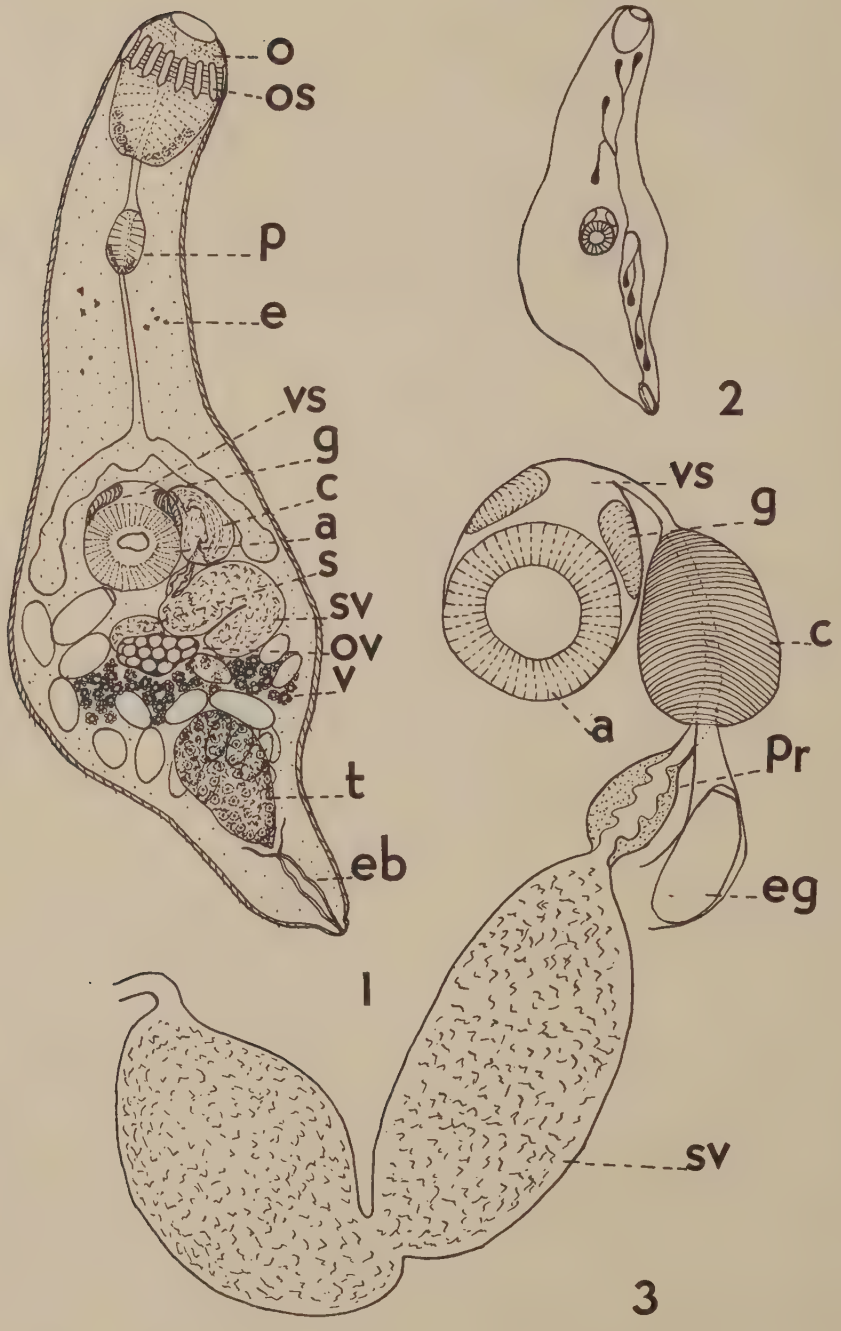
Genotype: Pygidiopsoides spindalis.

Pygidiopsoides spindalis n. sp.

(Figs. 1–3)

Diagnosis: With characters of the genus. Body spindle-shaped to oval and measuring 0.223–0.429 mm. (av. 0.314 mm.) in length and 0.066–0.095 mm. (av. 0.081 mm.) in maximum width. Cuticula moderately thick and covered with spines which decrease in size at both ends of the body. Fourteen spines ranging from 0.010 mm. to 0.012 mm. in length and 0.003 mm. in width surround the mouth. Circular muscles lie immediately mediad to circum-oral spines. Remnants of eyespots occur between pharynx and acetabulum. Oral sucker oval in lateral view and averaging 0.028 mm. wide and 0.049 mm. long. Prepharynx approximately as long as pharynx. Pharynx muscular, varying from spherical to oval, and measuring 0.016 mm. in diameter when spherical. Esophagus two to three times the length of the pharynx. Cecal bifurcation anterior to acetabulum, ceca terminate at or near the posterior margin of acetabulum. Acetabulum in mid-body region, enclosed in ventro-genital sac, and measuring 0.028–0.033 mm. (av. 0.030 mm.) in length and 0.025–0.028 mm. (av. 0.027 mm.) in width. Two lenticular gonotyls lateral and partially anterior to the acetabulum, enclosed in ventro-genital sac. The ventro-genital sac can be everted to expose the acetabulum and the gonotyls. The single testis is approximately 0.04 mm. in length and 0.024 mm. in width. Seminal vesicle voluminous, bipartite, and filled with spermatozoa. "Prostate" well-developed, with lobed cells projecting into its lumen. Ovary anterior and approximately one-third the size of the testis. Seminal receptacle nearly the same size as the ovary and dorsal to it. Laurer's canal opens on dorsal surface. Uterus in mature specimens occupies most of the posterior half of the body, and is filled with relatively large, operculate eggs which are nearly colorless when newly formed but become yel-

Received for publication, November 29, 1950.


o
os
p
e
vs
g
c
a
s
sv
ov
v
t
eb
1
2
vs
g
c
a
Pr
eg
sv
3

low in the more distal portions of the uterus. Eggs 0.026–0.028 mm. (av. 0.028 mm.) in length and 0.013–0.015 mm. (av. 0.014 mm.) in width. Older eggs contain ciliated, non-oculate miracidia. Common genital ejector very nearly the size of the acetabulum, with thick muscular walls exhibiting striations. Common genital canal leads from common genital ejector to ventro-genital sac. Excretory bladder tubular to spindle-shaped. Main excretory tubes pass anteriorly from the bladder to the mid-body region where they divide to form anterior and posterior branches each of which drains two pairs of flame cells (Fig. 2). The excretory pattern is represented by the formula $2[(2+2)+(2+2)]=16$.

Type specimen: Pygidiopsoides spindalis n. sp., deposited as number 503 in the Hancock Parasitology Collection.

Hosts: Second intermediate (natural): *Fundulus parvipinnis parvipinnis* (Girard).
Definitive (experimental): *Felis catus* and *Gallus domesticus.*
Geographic distribution: Southern California.

DISCUSSION

The genus *Pygidiopsoides* appears to be most closely related to *Pygidiopsis* Looss, 1907 the type species (*P. genata* Looss, 1907) of which was recovered from a pelican, *Pelecanus onocrotalus,* in Cairo, Egypt. It differs from *Pygidiopsis* and all other genera included in the subfamily CENTROCESTINAE Looss, 1899 in the possession of a large, muscular, common genital ejector. In living specimens, sperm and eggs were observed to enter this organ and then pass by way of a short canal to the ventro-genital sac. *Pygidiopsoides* has a more anterior location of the vitellaria, two gonotyls, and a single testis while *Pygidiopsis* has a posterior location of the vitellaria, one gonotyl, and two testes. *Pygidiopsoides* is placed in the subfamily CENTROCESTINAE Looss, 1907 which according to Price (1940) includes the following genera: *Centrocestus* Looss, 1899 (syns. *Stamnosoma* Tanabe, 1922; *Stephanopirumus* Onji and Nishio, 1924); *Ascocotyle* Looss, 1899; *Pygidiopsis* Looss, 1907; and *Phagicola* Faust, 1920 (syns. *Parascocotyle* Stunkard and Haviland, 1924; *Metascocotyle* Ciurea, 1933). Two genera not listed by Price notably should be added to those belonging to the CENTROCESTINAE. One of these, *Lacerdaia,* was described by Travassos (1931), the type specimen, *L. lacerdaia,* having been obtained from a marine shore bird, *Sula leucogastra,* captured in the vicinity of Rio de Janeiro. The other, *Caimanicola marajoara,* was described by Freitas and Lent (1938) from the intestine of the crocodilian, *Caiman sclerops* Gray, collected in Brazil. Much of the detail of the terminal genitalia is lacking in the descriptions of these two genera and in neither case is any mention made of the acetabulum being enclosed in a ventro-genital sac although it is likely that such is the case.

Characteristically the members of the subfamily CENTROCESTINAE have one or two complete or partially complete rings of fairly large circum-oral spines although some species, for example *Pygidiopsis genata* Looss, 1907, because of failure to observe or to loss due to handling, have been described without such spines. *Lacerdaia, Caimanicola, Centrocestus, Pygidiopsis,* and *Pygidiopsoides* have the normal type of oral sucker which is not prolonged posteriorly into a cone while the genera

EXPLANATION OF PLATE

ABBREVIATIONS USED: a acetabulum; c common genital ejector; e eyespot remnants; eb excretory bladder; eg egg; g gonotyl; o oral sucker; os circum-oral spine; ov ovary; p pharynx; pr prostate; s seminal receptacle; sv seminal vesicle; t testis; v vitellaria; vs ventro-genital sac.

FIG. 1. Ventral view of adult, *Pygidiopsoides spindalis.*
FIG. 2. Diagram of excretory system on one side of body.
FIG. 3. Diagram of terminal genitalia and associated organs.

Ascocotyle and *Phagicola* have a caudal appendage on the oral sucker. Although Witenberg (1929) considered valid the genus *Parascocotyle* Stunkard and Haviland, 1924, Price (1932, 1935) on the basis of a restudy of Faust's specimens in which he determined a number of details inadequately reported in the original description of *Phagicola pithecophagicola Faust,* 1920, considers *Parascocotyle* a synonym of *Phagicola.*

Probably the cercariae of all these genera possess eyespots since Chen (1948) described an eyespotted cercaria for *Centrocestus formosanus* (Nishigori, 1924) and remnants of eyespots have been observed in the adults of *Pygidiopsis phalacrocoracis* Yamaguti, 1939 and *Pygidiopsoides spindalis.*

SUMMARY

A new genus and species of heterophyid trematode, *Pygidiopsoides spindalis* is described from southern California, U.S.A.

The natural second intermediate host is the fish, *Fundulus parvipinnis parvipinnis* (Girard).

The experimental definitive hosts are *Felis catus* and *Gallus domesticus.*

The excretory pattern is represented by the formula $2[(2+2)+(2+2)]=16$.

LITERATURE CITED

CHEN, H. T. 1948 Some early larval stages of *Centrocestus formosanus* (Nishigori, 1924). Lingnan Sci. J. **22**: 93–104.

FAUST, E. C. 1920 Notes on trematodes from the Philippines. Philipp. J. Sci. **17**: 627–634.

FREITAS, J. F. T. AND LENT, H. 1938 Pesquisas helminthologicas realisadas no Estado do Parã. 11. Dois novos trematodeos de *Caiman sclerops* Gray. Mem. Inst. Oswaldo Cruz **33**: 53–56.

LOOSS, A. 1899 Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. Zool. Jahrb., Syst. **12**: 521–784.

———1907 Notizen zur Helminthologie Aegyptens. VII. Ueber einige neue Trematoden der ägyptischen Fauna. Centrbl. Bakt. Orig. **43**: 478–490.

PRICE, E. W. 1932 On the genus *Phagicola* Faust. J. Parasit. **19**: 88–89.

———1935 Descriptions of some heterophyid trematodes of the subfamily Centrocestinae. Proc. Helm. Soc. Wash. **2**: 70–73.

———1940 A review of the trematode superfamily Opisthorchioidea. Proc. Helm. Soc. Wash. **7**: 1–13.

STUNKARD, H. W. AND HAVILAND, C. B. 1924 Trematodes from the rat. Amer. Mus. Novit. No. 126: 10 pp.

TRAVASSOS, L. 1931 Contribuições ao conhecimento das Heterophyidae (Trematoda). Mem. Inst. Oswaldo Cruz **25**: 47–49.

WITENBERG, G. 1929 Studies on the trematode family Heterophyidae. Ann. Trop. Med. Parasit. **23**: 131–239.

YAMAGUTI, S. 1939 Studies on the helminth fauna of Japan. Pt. 25. Trematodes of birds, IV. Jap. J. Zool. **8**: 129–210.

LEUCOCYTOZOA AND OTHER BLOOD PARASITES OF THE PURPLE GRACKLE, *QUISCALUS QUISCALA QUISCALA*

REGINALD D. MANWELL*

Although the avian malarial parasites are becoming increasingly well known, there is still little knowledge of most of the other blood parasites of wild birds. This is true both with regard to the parasites themselves, and to their incidence and effects on given host species, even though a number of parasite surveys have been made in recent years.

In many respects, this field of investigation is one of the most inviting in parasitology at the present time. The potential importance of any avian parasites which may be pathogenic is obvious because of the significant role birds play in the maintenance of biological balances, and in agriculture. Some species of birds are important to the sportsman. Parasitic genera, such as *Haemoproteus* and *Leucocytozoon,* have an added interest because of their undoubted relationship to the malarial plasmodia of man and animals.

For these reasons the author has been interested in the blood parasites of birds for many years. Traps have been operated in or near Syracuse (at Fayetteville),

TABLE 1.—*Blood Parasites of the Grackle*

Authority and Locality	Grackle subspecies	Total Cases	Negative	Malaria, Plasmodium: *cath.*	*circ.*	*elong.*	*hex.*	*rel.*	*und.*	Other Blood Parasites: *Haem.*	*Leu.*	*Mf.*	*Tox.*	*Trp.*
This study; Syracuse, N. Y.	*Q. q. quiscala*	75	19	3	0	0	0	1	8	30	43	0	0	0
Herman, 1938; Cape Cod, Mass.	?	31	30	0	0	0	0	0	1	0	0	?	0	0
Huff, 1939; mostly Kan., Ill.	*Q. q. aeneus*	about 63 (Q.q.) 128 (aeneus)		0	0	3	0	3	7	45	4	2	?	1
Jordan, 1943; Athens, Ga.	?	3	1	0	0	0	1	1	0	1	0	0	?	?
Wetmore, 1941 D.C.	*Q. q. quiscala*	89	34	0	0	0	0	0	14	21	41	5	7	2
same; same	*Q. q. aeneus*	1	0	0	0	0	0	0	0	1	1	0	0	1

Abbreviations: Species of malaria: "cath." *cathemerium*, "circ." *circumflexum*, "elong." *elongatum*, "hex." *hexamerium*, "rel." *relictum*, "und." undetermined. Other blood parasites: "Haem." *Haemoproteus*, "Leu." *Leucocytozoon*, "Mf." Microfilaria, "Tox." *Toxoplasma*, "Trp." *Trypanosoma*.

N. Y., and the blood of all birds caught has been examined for microorganisms. Sometimes a series of such examinations has been possible, since some species are often recaught after liberation. Certain species are also much more easily trapped than others, among them the purple grackle which forms the subject of the present study. This species has several advantages as a host for the study of both naturally and experimentally acquired infections. It is large, easily maintained in captivity, and susceptible to most of the main types of blood parasites (as may be seen from Table 1 below). It is also easily retrapped so that changes in the incidence of various kinds of parasites from one time to another are easily followed.

Received for publication, October 24, 1950.

*From the Department of Zoology, Syracuse University. This study was aided in part by grants from the National Institute of Health and the American Philosophical Society.

The data in this study were obtained from the microscopical study of blood and organ smears. The former were made as soon as birds were brought into the laboratory, and were stained with the J.S.B. (Jaswant/Singh and Bhattacharji, 1944) stain (Manwell and Feigelson, 1948). All blood smears were examined for a minimum of 5 minutes under oil. Organ smears were made from certain birds bearing heavy infections of *Leucocytozoon,* especially when large numbers of developmental stages were present. Contact preparations were employed in preference to sections, because of the saving of time, and also because they are superior in some respects when a search for developmental stages is to be made. The J.S.B. stain is equally good for such preparations, being very rapid and convenient and giving excellent differentiation.

OBSERVATIONS

Leucocytozoon was by far the commonest parasite encountered, but it was soon discovered that the incidence of *Haemoproteus* was almost as high. When only one of these two parasites was at first seen, more search would very often reveal the other. This was a complicating factor in the study of the tissue stages of *Leucocytozoon,* as indicated below.

The incidence of these and other blood parasites is shown in Table 1, together with the corresponding figures obtained by other investigators. The only other extensive and strictly comparable study is that of Wetmore (1941), made in the District of Columbia, and her results parallel those herein reported. Huff (1939) found a similar incidence of *Haemoproteus,* but few cases of *Leucocytozoon.* However all the grackles he studied belonged to the subspecies *Quiscalus quiscalus aeneus* rather than to *Quiscalus quiscalus quiscalus,* and "by far the greatest proportion" of the smears were from Kansas, Ill. It is very possible that the incidence of a given parasite may vary, not only in different host species or subspecies, but also geographically.

Thus Herman (1938) studied 31 grackles caught on Cape Cod, but failed to find *Haemoproteus* or *Leucocytozoon* in any of them (he failed to state the subspecies to which they belonged). It is of course quite possible that the incidence of these and other parasites varies with the season, but there is little reason to think that the varying results of different surveys can be explained in this way, especially as most blood protozoa seem to produce very chronic infections. Acute infections may however be more common in very young birds. It is also rather unlikely that the methods of different investigators vary enough to greatly influence relative results, at least for *Leucocytozoon* and *Haemoproteus,* since it is the author's experience that such infections are rather quickly detected from blood smears.

Few infections with blood parasites other than these two were found among grackles. Malaria occurred infrequently, and the only species found were *Plasmodium cathemerium* and *P. relictum.* Apparently no one has yet seen other species of *Plasmodium* in this host, except *P. elongatum* (seen by Huff), and *P. hexamerium* (reported by Jordan).

No cases of trypanosomiasis, filariasis, toxoplasmosis, or haemogregarine infection were observed.

After it was found that *Leucocytozoon* was very common among grackles, search

was made for the reproductive stages and attention was very soon directed to the kidneys because of the tremendous concentration of developing and mature gametocytes often observed there (Figure 1). In certain cases, such parasites were almost as numerous as the erythrocytes. *Haemoproteus,* when present, was never more numerous there than in the blood.

Although a number of kidney slides were studied without success, multiplicative forms believed to be of *Leucocytozoon* were finally found in quantity on some. That they might have been stages in the life-cycle of *Haemoproteus* is rendered very unlikely not only by the very small numbers of such gametocytes present in the blood (when any at all were seen), but also by the complete absence of young parasites in such cases, and by the fact that in such species of *Haemoproteus* as have been carefully studied, multiplicative stages in the vertebrate host are confined to the lungs.

Malaria may also be ruled out. It is not only rare in grackles, but no such parasites were found in either blood or organ smears, and no evidence of malarial pigment was seen. The time given to the study of blood and tissue smears of *Leucocytozoon*-infected birds usually amounted to a number of hours, so that there was little possibility of missing blood parasites of any kind.

The morphology of the multiplicative forms seen in the kidneys is shown in Figures 2 to 6. Apparently they are most numerous about the tubules, and appear to have a quite local distribution. The youngest forms are minute bits of protoplasm which very early exhibit nuclear division. A moderately young stage is shown in Figure 2. Growth in size appears to go hand in hand with continued division of the chromatin, until relatively enormous segmenting stages result (Figure 6). As far as can be determined from smears alone, lymphocytes are the preferred type of host cell for the gametocytes, but two young parasites were seen in what appeared to be a monocyte. There is no reason for thinking that in this species "there is no blood cell parasitized" as claimed by Johnson (1945) to be the case in *Leucocytozoon smithi* of turkeys. The sexual forms in the grackle, however, all appeared to be extra-cellular, and they probably correspond to the "megaloschizonts" described by Huff (1942) from the duck, and by Wingstrand (1947a and b) from the Swedish crow. Nothing which could be homologized with the "hepatic schizonts" of these investigators was seen, although one rather small schizont was seen, free in a liver smear.

A point of interest is the appearance of vacuoles in all the schizonts and segmenters. These appearances could hardly have been due to post-mortem changes or beginning degeneration, since the preparations were made immediately after sacrifice of the bird. Presumably the vacuoles represent the "cleft-like vacuoles" seen by Huff and Wingstrand in their sections. Similar structures were noted by the first of these two authors in the tissue stages of *Plasmodium relictum* var. *matutinum,* and were also described in an earlier paper (Manwell, 1940).

A second feature worth noting is the relatively great size of the larger schizonts, because in this respect they seem to surpass either *Haemoproteus* or *Plasmodium.* Yet none of the schizonts appeared fully grown, and no clusters of merozoites were observed. The larger schizonts showed a remarkable tendency to pull out into strands of varying shapes and dimensions (Figure 6).

DISCUSSION

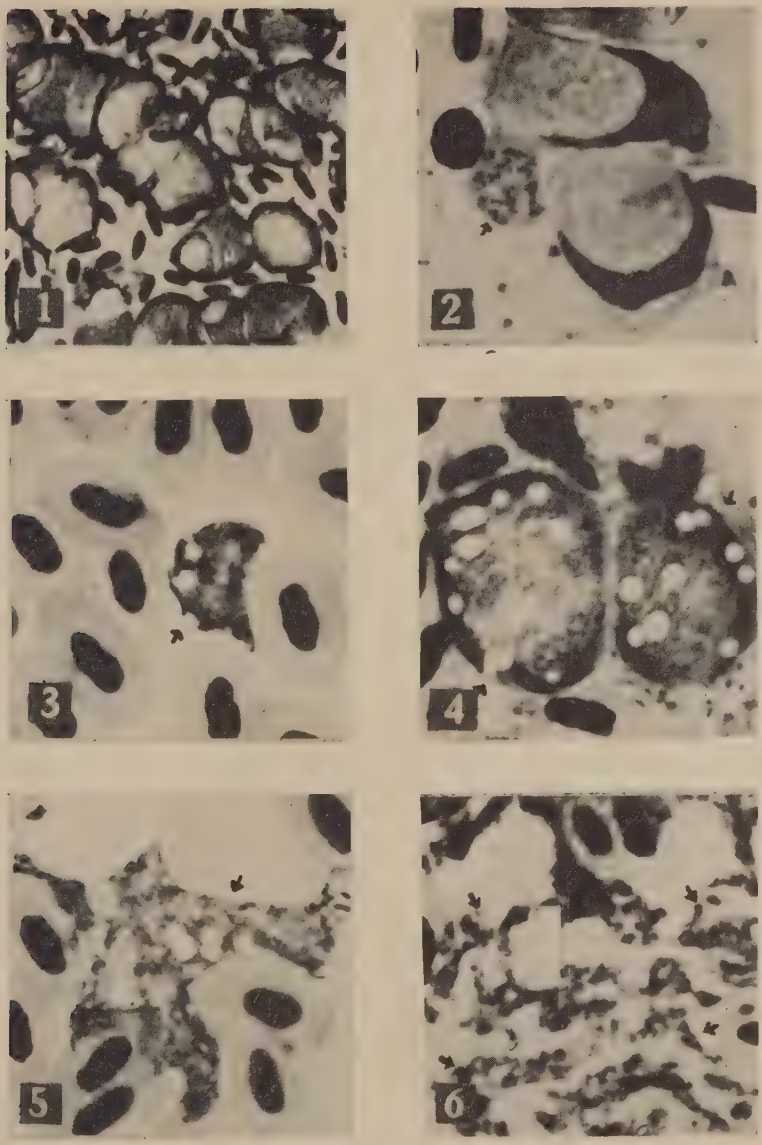
The conflicting nature of the data in Table 1 emphasizes how little is certainly known about the blood parasites of even a very common species of wild bird, such as the grackle. Almost any piece of research of this nature is likely to turn up something new. We have few facts about the relative importance of the various avian parasites in nature, or of their vectors and life histories. The divergent results of previous avian parasite-surveys (Table 1) make even clearer the gaps in what we know in this field.

So little is known about the genus *Leucocytozoon* that it is not yet possible to define just what constitutes a valid species. Though many species have been named, most of them rest on nothing more substantial than a supposed, but not demonstrated, host-specificity. The same thing is true of *Haemoproteus*. It seems to the author that new species within these genera should not be created unless (1) there are morphological characteristics definite and constant enough to make specific identification readily possible from study of the gametocytes alone, or (2) enough is known about the life-cycle to make it certain that what is to be called a species is unique in some respect, or (3) it is certain that what is believed to be a distinct species is in fact specific for a given species of host. For this reason I am not attempting to give a specific name to either the *Haemoproteus* or the *Leucocytozoon* of the grackle. Huff (1939) and Wetmore (1941), who appear to have been the first to find these two parasites in the bronzed and purple grackle respectively, also refrained from giving specific names, probably for like reasons.

It is also appropriate to point out in passing that the vectors of most leucocytozoa are still entirely unknown. Species of *Simulium* (black flies or buffalo gnats) have been incriminated by Skidmore (1932), O'Roke (1934), and Johnson, Underhill, Cox, and Threlkeld (1938) for the leucocytozoa of ducks and turkeys, but it is at least possible that for the leucocytozoa of other host species, the vectors may belong to other genera of biting insects. It is of interest that no external parasites, other than a single tick, were found on the nearly 100 grackles brought into the laboratory. It is also true that the vectors of most species of *Haemoproteus* are still unknown, and that they may not all be hippoboscid flies.

It is likely that when the evidence is all in, it will be found that one of the characteristics of species of *Leucocytozoon,* and very probably of *Haemoproteus* as well, is the tissue or organ of the body preferred for asexual multiplication. Thus, in the studies of Huff and Wingstrand, parasites were especially abundant in the liver and spleen. In the grackle, the kidney is apparently the organ of first choice. But, in a number of other host-species which the author has examined (including the robin, cowbird, Canada Jay, field sparrow, song sparrow, and white-crowned sparrow) no multiplicative stages and no concentration of gametocytes have been found in the kidney.

A related problem of great interest is that of just what it is about a given species of parasite and a given tissue or organ which makes the latter especially suited for the physiological needs of the former. If we only knew the precise answer to this question, much light might be cast on a number of other problems, such as the real nature of a species, relationships of species to one another, the factors required for *in vitro* cultivation, and even of parasite genetics.



EXPLANATION OF PLATE I

FIG. 1. Shows the great concentration of gametocytes in the kidney. The field is chosen almost at random.

FIG. 2. Two sexual forms and a young schizont (arrow).

FIG. 3. A somewhat larger schizont, with the characteristic vacuoles.

FIG. 4. Two moderately large schizonts, each showing numerous vacuoles.

FIG. 5. A more advanced schizont, with the usual vacuoles. Note the tendency to be pulled out of shape.

FIG. 6. A portion of a very large schizont (almost a segmenter), showing the stranded appearance which many of them exhibit. (Arrows indicate main boundaries of parasite.)

Note: All the microphotographs were made by Miss Stella Zimmer of the Medical School of the University of New York, at Syracuse University (Department of Photography). The magnification of Figure 1 is 790×, of all other figures, 1800×. The preparations were all contact preparations, stained with the J.S.B. stain.

SUMMARY AND CONCLUSIONS

Haemoproteus and *Leucocytozoon* have been found to be the most common blood parasites of the purple grackle (*Quiscalus quiscala quiscala*). A few cases of *Plasmodium relictum* and *P. cathemerium* were also demonstrated.

Organ smears of certain of the birds with the heaviest infections of *Leucocytozoon* revealed great numbers of gametocytes in the kidneys, and associated with them, many schizonts of various degrees of development. No similar reproductive forms, nor any such concentrations of gametocytes, were seen in any other organ. The schizonts bore a strong resemblance to corresponding stages in the asexual cycle of *Haemoproteus* and *Plasmodium*, but they are believed to be the multiplicative forms of *Leucocytozoon*. It is suggested that descriptions of species of *Leucocytozoon* should include information about the tissue or organ in which multiplication in the vertebrate host takes place and when possible, of course, the species of vector. Specific determination based chiefly on a supposed host-specificity, or even upon the morphology of the gametocytes, cannot be considered enough.

REFERENCES

HERMAN, CARLTON M. 1938 The relative incidence of blood protozoa in some birds from Cape Cod. Trans. Amer. Micros. Soc. **57**: 132–141.

HUFF, CLAY G. 1939 A survey of the blood parasites of birds caught for banding purposes. Jour. Amer. Vet. Med. Ass. **94**: 615–620.

———1942 Schizogony and gametocyte development in *Leucocytozoon simondi*, and comparisons with *Plasmodium* and *Haemoproteus*. Jour. Inf. Dis. **71**: 18–32.

JOHNSON, E. P. 1945 Blood parasites of turkeys. Mich. St. Coll. Vet. **5**: 145–147.

———, UNDERHILL, G. W., COX, J. A., AND THRELKELD, W. L. 1938 A blood protozoon of turkeys transmitted by *Simulium nigroparvum*. Amer. Jour. Hyg. **27**: 649–665.

JORDAN, HELEN B. 1943 Blood protozoa of birds trapped at Athens, Georgia. J. Parasit. **29**: 260–263.

MANWELL, REGINALD D. 1940 Life-cycle of *Plasmodium relictum* var. *matutinum*. Amer. Jour. Trop. Med. **20**: 859–867.

——— AND FEIGELSON, PHILIP 1948 A modified method of preparing the J.S.B. stain. Jour. Lab. and Clin. Med. **33**: 777–782.

O'ROKE, E. C. 1934 A malaria-like disease of ducks. Univ. Mich. School of Forestry and Conservation, Pub. 4.

SINGH, JASWANT AND BHATTACHARJI, L. M. 1944 Rapid staining of malarial parasites by a water-soluble stain. Ind. Med. Gaz. **79**: 102.

SKIDMORE, L. V. 1932 *Leucocytozoon smithi* infection in turkeys and its transmission by *Simulium occidentale*, Townsend. Centralbl. Bakt., Orig. B, **125**: 329–335.

WETMORE, PSYCHE W. 1941 Blood parasites of the District of Columbia and Patuxent Research Refuge vicinity. J. Parasit. **27**: 379–393.

WINGSTRAND, K. G. 1947a On some Hematozoa of Swedish birds with remarks on the schizogony of *Leucocytozoon sakharoffi*. Kungl. Svenska Vetenskapsakad., Handl., Stockholm, **24**: 31 pages.

———1947b Further studies on *Leucocytozoon sakharoffi*. Kungl. Svenska Vetenskapsakad. Handl., Stockholm, **24**: 17 pages.

MULTIPLE HELMINTHIC INFECTIONS

Walter Koch and Heinz Steinitz

The Hebrew University, Jerusalem

In a survey published elsewhere (Yoeli et al., 1949) it had been noted that the two groups[1] of Jewish population differed in their rate of helminthic infection. The significance of this difference can be readily estimated from a fourfold table (Fisher, 1946, Fisher and Yates, 1943), viz.:

Table 1

	Infected with Worms	Not infected with Worms	Total
Orientals	83	64	147
Ashkenasics	75	228	303
	158	292	450

Applying the chi-square test to this table it is found that the rates in Orientals and Ashkenasics differ significantly (p less than 0.001). When pooling the two communities and grouping according to sex, the rate among females is higher (0.445) than that among males (0.348). The difference between these rates just reaches the level of significance ($p = 0.032$).

The rate of simultaneous infection with *Ascaris lumbricoides* and *Trichuris trichiura* should be the product of *Ascaris* rate times *Trichuris* rate, or: $0.131 \times 0.308 = 0.0403$. Thus, among a population of 496 $0.131 \times 0.308 \times 496 = 20$ cases of simultaneous infections would be expected; the number actually found (40) is twice as high as expected. Again, from a chi-square test on a fourfold table it is found that this difference is highly significant (p less than 0.001). When grouping these double infections according to community and sex, significance is no longer attained; this might be due to the smallness of numbers.

Using the recent material of Boventer (1947) and Poindexter (1949) we calculated the expected rates of double infections with *Ascaris* and *Trichuris* from the numbers of persons infected with either worm. The actual numbers of double infections with both worms, as given by these authors, were lower or almost equal to those predicted. Boventer found among 2201 people 42.3% infected with *Ascaris* and 14.5% infected with *Trichuris;* his percentage of double infections (6.9) very closely approaches the predicted percentage of 6.1; a similar fit holds good for both of his sub-groups, Sicily and Italy proper. Poindexter's figure of 9% double infections, based on a population of 4000, is lower than the calculated one (13.2%). At present no explanation can be given why in our material the number of double infections found is twice as high as expected. An analysis performed on a greater population might give some clue as to the cause of this discrepancy.

Received for publication, October 2, 1950.

[1] Oriental Jews are of Mediterranean and Asiatic extraction. Ashkenasic is a term loosely used for Jews of Eastern European extraction. Of a total population of 496, 147 were Orientals, 303 Ashkenasics while the remaining 46 persons could not be classified.

References

Boventer, K. 1947 Ueber die Verbreitung menschlicher Eingeweidewürmer in Italien. Zbl. Bakt., I. Abt. Orig. **152**: 292–298; (Quoted from Trop. Dis. Bull., vol. 47, No. 3. p. 251 (1950).

Fisher, R. A. 1946 Statistical Methods for Research Workers. Tenth Edition, p. 78. Oliver and Boyd (Edinburgh).

———and Yates, F. 1943 Statistical Tables for Biological, Agricultural and Medical Research. Second Edition, p. 4. Oliver and Boyd (Edinburgh).

Poindexter, H. A. 1949 A Laboratory Epidemiological Study of certain Infectious Diseases in Liberia. Amer. J. Trop. Med. **29**: 435–442.

Yoeli, M., Steinitz, H., Gerichter, C. and Rabinowicz, K. 1949 The Incidence of Intestinal Infections in Kitchen-Personnel of the Army. Harefuah (Jerusalem), vol. 37, No. 7, (In Hebrew p. 83–4, English summary p. 84). cf. Trop. Dis. Bull., vol. 47, No. 1, p. 37, (1950).

PARASITOLOGICAL STUDY ON THE ESKIMOS IN THE KOTZEBUE AREA OF ALASKA

DOROTHY J. HITCHCOCK*

Consultant, Arctic Health Research Center, Public Health Service, F. S. A., Anchorage, Alaska

During the summer of 1950, a study of intestinal parasites was conducted at the Alaska Native Service Hospital, Kotzebue, Alaska. In the complete study, 376 different people were examined by one method or all. They came from 19 villages within the area surrounding Kotzebue and extending from Point Hope to St. Michael, and from Little Diomede Island to Shungnak, and ranged in age from 7 months to 75 years. The group included both inpatients from the general hospital and individuals who came for clinical care or x-raying. The only qualifying factor in patient selection was their willingness to submit six fecal samples for examination.

Six fecal samples from each of 100 Eskimos were examined. The procedures used were the same as in the Bethel study (Hitchcock, 1950) except that a direct saline smear examination was made only on soft specimens.

Perianal pinworm swabs (Jacobs, 1942) were taken from the hospital patients in the morning before bathing or evacuation, and from the outpatients between nine and eleven o'clock in the morning when they brought in their fecal samples. The "Scotch tape" swabs from the 108 persons were examined until eggs were found or until six swabs per person were examined.

The skin test for trichina was carried out on 300 Eskimos using *Trichinella* extract (Lederle). The readings were observed after 15–20 minutes.

TABLE 1.—*Prevalence of Parasitism in the Kotzebue Area, Alaska*

Parasite	No. of Persons Tested	Percentage Infected
Endamoeba coli	100	44
Endolimax nana	100	5
Iodamoeba bütchlii	100	2
Giardia lambl a	100	1
Diphyllobothrium sp.	100	6
Enterobius vermicularis	100	43
Trichinella spiralis, skin test reactors	300	1.6
Echinococcus granulosus, skin test reactors	366	3.2

The skin test for hydatid disease was performed on 366 Eskimos using *Dipylidium caninum* diagnostic antigen (Lederle). The readings were observed after 8–10 minutes.

The parasites which were recovered and the percentage findings are given in Table 1. The absence of *Endamoeba histolytica* conforms with the low incidence in the Bethel area. The incidence of *Diphyllobothrium* was less than half that found in the Bethel area. The Eskimos in the Bethel area consume more fresh water fish since many of the villages were located along the Kuskokwim River. In the Kotzebue area the native food consists of white whale, reindeer, bear, and fish, chiefly

Received for publication, October 19, 1950.

* Instructor of Parasitology, Department of Bacteriology and Public Health, Michigan State College, East Lansing, Michigan.

of the salt water variety. The only nematode eggs observed were from the human pinworm, *Enterobius vermicularis*. In the examination of 264 stools from 47 people shown to be infected by the swab technique, only 4 diagnoses would have been made by the $ZnSO_4$ method, and one by the Na_2SO_4-triton-ether method. Adult pinworms were not recovered by any of the methods used. In two cases of *Diphyllobothrium* infection, the eggs were recovered and identified on the "Scotch tape" swabs.

Classification by sex of the 108 people included in the pinworm study revealed that of 32 males examined, 14 or 44% were infected; of 76 females examined, 33 or 43% were infected. No significant difference in the rate of infection was found according to age. Of 52 individuals in the age group, 7 months to 22 years, 43% were found to be infected, and of 56 in the age group 22 to 75 years, 45% were infected.

Intradermal skin tests for trichina and hydatid infections were carried out on Eskimos ranging in age from 4 to 75 years. Of the 300 people tested for trichina, 5 or 1.6% were positive reactors. One gave a history of having eaten raw pork and two had eaten raw bear, seal, and whale. The information given by two children was questionable.

Of the 366 people skin tested for hydatid disease, 12 or 3.2% were reactors. Of these 12, nine were from 13–26 years of age. Fecal samples could not be obtained from all reactors as several individuals were in Kotzebue for only a day. Since *Diphyllobothrium latum* was the only cestode found in the examination of 200 Eskimos in the combined Bethel and Kotzebue areas, this reaction is not likely to be a cross reaction caused by any other tapeworm. The six cases of *Diphyllobothrium* infection found in the Kotzebue area showed no reaction to the hydatid skin test. Hydatid skin testing was not carried out in the Bethel area.

Several of the reactors to the hydatid skin test at Kotzebue were known to have advanced pulmonary tuberculosis. In order to rule out the possibility of false reactions due to tuberculosis, the same skin test was administered to 28 patients with advanced pulmonary tuberculosis at the Ingham County Tuberculosis Sanatorium, Lansing, Michigan, who had not been out of the United States. None of these patients reacted to the hydatid skin test.

The only suggestion of a fluke infection was obtained from one man who came to the clinic with gastro-intestinal symptoms. By means of the Na_2SO_4-triton-ether method, a very few yellowish-brown, operculated, non-embryonated eggs, measuring 77 μ by 84 μ, were recovered from only one of the six stools examined. Three morning sputums did not contain these eggs. At the time of finding the eggs the patient gave a history of having eaten cooked and dried fish.

The geographic distribution of the parasites recovered showed no evidence of localization except in the instance of *Diphyllobothrium,* 4 of the 6 cases having came from Selawik.

As in the previous study at Bethel, the Eskimos were most cooperative. The writer wishes to express her appreciation to the many individuals who assisted in the study. Special thanks are due Dr. S. E. Rabeau and the staff of the Alaska Native Service Hospital at Kotzebue, and of the Arctic Health Research Center at Anchorage, for their help in making this study possible.

SUMMARY

1. Examination of six fecal samples from each of 100 Eskimos failed to reveal *Endamoeba histolytica*. The incidence of the non-pathogenic intestinal protozoa was about the same as in the Bethel study.

2. *Diphyllobothrium sp.* was found in 6% of the group examined. In the Bethel group the incidence was 15%.

3. *Enterobius vermicularis* was found in 43% of the people examined, occurring about equally in children and adults.

4. Of the 300 Eskimos skin tested for trichina 1.6% were positive reactors, and 3.2% of the 366 Eskimos skin tested for hydatid disease were positive reactors.

References

Hitchcock, D. J. 1950 Parasitological study on the Eskimos in the Bethel area of Alaska. J. Parasit. **36:** 232–234.

Jacobs, A. H. 1942 Enterobiasis in children, incidence, sympthomatology and diagnosis with a modified Scotch cellulose tape technique. J. Pediat. **21:** 497–503.

TWO ANOPLOCEPHALID CESTODES, *CITTOTAENIA PRAECOQUIS* STILES AND *CITTOTAENIA MEGASACCA* N. SP., FROM THE WESTERN POCKET GOPHER, *THOMOMYS TALPOIDES*, OF WYOMING*

CHARLES F. SMITH
University of Nebraska, Lincoln

During the summer of 1948, numerous cestodes were found in the small intestine of one of several pocket gophers examined. This host, *Thomomys talpoides clusius*, was taken 8 miles north and 19½ miles east of Savoy, Carbon County, Wyoming. The cestodes were killed and preserved in 5% formalin solution. Whole mounts and frontal serial sections, cut at a thickness of 35 μ, were made of each of the two species in the collection. Toto mounts were stained in Delafield's haemotoxylin; sections were stained with Delafield's and counterstained with eosin.

Both species are anoplocephalids of the genus *Cittotaenia;* one of them apparently a new species in that genus.

Family Anoplocephalidae Fuhrmann, 1907
Sub-family Anoplocephalinae Blanchard, 1891
Cittotaenia Riehm, 1881

The genus *Cittotaenia* was characterized by Baer (1927) as follows: Anoplocephalidae of medium size. Sexual pores double. Two sets of reproductive organs per segment. Sex ducts pass dorsally to the excretory vessels and nerve. The excretory system forms 4 longitudinal tubes, possibly in some cases presenting an anastomosing accessory system. Testes in a single clump or in 2 groups. Cirrus pouch well developed. The uterus is a simple or double transverse tube which may have elongations or become reticular. It may pass the excretory vessels laterally on the dorsal face. Eggs are provided with pyriform apparatus. Adults in rodents and birds. Type species: *Cittotaenia denticulata* (Rudolphi, 1804) Stiles, 1896.

Cittotaenia praecoquis Stiles, 1895
Syn. *Ctenotaenia praecoquis* Stiles, 1895
Cittotaenia bursarius (Stiles, 1895) Stiles and Hassall, 1896

The following data are based on 12 complete specimens plus fragments of other specimens.

The scolex is 0.74 to 0.84 mm. in diameter. The circular suckers are 0.26 to 0.29 mm. in outside diameter, and the walls are 0.065 mm. in thickness. There is no neck and incomplete segmentation is seen immediately posterior to the scolex.

The total length of the strobila varies from 75 to 150 mm. The average width is 2.0 mm. The number of proglottids varies from 176 to 262, the average being approximately 200. The mature proglottids are five times as wide as long and the gravid proglottids are twice as wide as long.

The genital anlagen appear before segmentation is complete. The ovary is seen developing first and the testes shortly thereafter. The proglottids become mature between the 40th and 65th proglottid. The uterus first appears between the 130th and 145th proglottid. The genital organs thereafter quickly atrophy until only the uterus remains as a sac containing the eggs which fill almost the entire proglottid.

The smaller dorsal excretory vessel is 0.015 mm. in diameter. The larger ventral excretory vessel is 0.05 mm. in diameter and is ventral and lateral to the former.

The 45 to 60 testes per segment are ovoid and measure 0.039 to 0.054 mm. They are dorsal to the other genital organs but they do not extend beyond the excretory vessels on either side. The vas deferens is coiled and in most cases enlarges to form an external seminal vesicle. The cirrus sac is pyriform and measures 0.09 mm. in diameter and is 0.24 mm. in length. It passes dorsally to the excretory vesssels.

The two ovaries per segment are multilobed, fan-shaped structures composed of 15 to 20 lobes. A seminal receptacle is situated at the base of each ovary. The vagina passes dorsally

Received for publication, November 29, 1950.

* Studies from the Department of Zoology, University of Nebraska, No. 252. The work was done under the direction of Dr. H. W. Manter.

to the excretory vessels. The uterus is at first a transverse tube in the anterior, ventral part of the proglottid, extending laterally past the excretory vessels. It develops outpocketings in all directions but becomes saccate in the gravid proglottid.

The eggs have the typical pyriform apparatus with filaments and are encased in a shell 0.035 mm. in diameter. The onchosphere is 0.009 mm. in diameter.

Two specimens of *C. praecoquis* were noted in which some proglottids did not have double sets of reproductive organs. In one specimen, 13 proglottids were found with single sets of reproductive organs. These 13 were not at any regular interval, nor were the reproductive organs always on the same side. However, 4 of the 13 were successive proglottids. In the other specimen, there were six proglottids at irregular intervals which contained only one set of reproductive organs.

Host: Thomomys talpoides clusius, Coues pocket gopher.

Location: Small intestine.

Locality: Carbon County, Wyoming.

Cittotaenia megasacca n. sp.
(Figs. 1–5)

The following data are based on the study of 10 complete specimens.

Description: The scolex is 0.65 to 0.82 mm. wide by 0.65 mm. long. The anterior end can be invaginated into the posterior portion of the scolex which may act as a protective covering. The four oval suckers are well developed and open laterally. They measure 0.22 by 0.19 mm., outside diameter, and the muscular walls are 0.04 mm. in thickness. There is no neck and segmentation begins immediately.

The total length of the strobila varies from 14 to 22 mm. The greatest width, which is attained approximately one-fifth of the length from the posterior end, is 4 mm. Mature proglottids are approximately 12 times wider than long; older proglottids become longer and less wide, until the terminal segment is approximately only three times as wide as long.

The number of proglottids is from 68 to 89, averaging 74 per strobila. The genital anlagen, both male and female appear in the first segment. Proglottids become mature in the 12th segment, gravid in the 30th. The posterior half of the entire worm is composed of gravid proglottids. Apparently the development and atrophy of the genital organs are accomplished with great rapidity.

The smaller dorsal excretory vessel is 0.019 mm. in diameter and the larger ventral excretory vessel is 0.32 mm. in diameter and is ventral and slightly median to the former.

The 100 to 135 testes per proglottid are ovoid and measure from 0.025 to 0.067 mm. in diameter. The majority of the testes are dorsal to the other genital organs. In mature proglottids at least some testes are situated laterally beyond the excretory vessels. The vas deferens is coiled and in most cases enlarges to form an external seminal vesicle. The cirrus sac is pyriform and measures 0.15 mm. in diameter at its largest part and may be 0.52 mm. in length. There are a large internal seminal vesicle and prostate gland cells within the cirrus sac. The unarmed cirrus may attain a length of 0.40 mm. and is 0.02 mm. in diameter. The cirrus sac passes dorsally to the excretory vessels.

The two ovaries which are situated in the middle of each lateral half of the proglottid, are fan-shaped structures composed of from 25 to 36 lobes. The globular vitellarium is situated at the posterior edge of each fan-shaped ovary. The seminal receptacle is posterior and slightly dorsal to the base of the ovary in the very young mature proglottids. The seminal receptacle rapidly increases in size in older proglottids to a size 0.25 by 0.80 mm. It is by far the most conspicuous organ in the mature proglottids. The vagina passes dorsally to the excretory vessels on each side and is ventro-posterior to the cirrus sac. The uterus is at first a transverse tube in the ventro-anterior part of the proglottid and extends ventrally to, and laterally past the excretory vessels. It soon develops outpocketings in all directions and in the gravid proglottids it is saccate and almost completely filled with eggs.

Genital pores are lateral and approximately at mid-proglottid level.

The eggs have the typical pyriform apparatus of the embryophore and are encased in a shell 0.038 mm. in diameter. The onchosphere is 0.01 mm. in diameter.

The name *megasacca* is from *megas* = large and *saccus* = sac, and refers to the large seminal receptacle in the mature proglottids.

Host: Thomomys talpoides clusius, Coues pocket gopher.

Location: Small intestine.

Locality: Carbon County, Wyoming.

The type specimen will be deposited with the U. S. National Museum, No. 37188.

DISCUSSION

Cittotaenia megasacca is unusually thick and the usual clearing solutions, i.e., xylol, cedar-wood oil and glycerin, did not clear the stained specimens enough to allow satisfactory study of the internal morphology. Frontal sections cut 35 μ thick, of an entire specimen, were very useful and permitted satisfactory study of the internal morphology. *C. praecoquis,* on the other hand, readily cleared with xylol and the stained whole mounts showed clearly the internal morphology.

Several keys to the species of *Cittotaenia* have been published. In these, *C. megasacca* would key to *C. praecoquis,* which is probably the most closely related species. *C. megasacca* differs from *C. praecoquis* in the following respects: it has a maximum length of 25 mm. compared with 150 mm.; it has a maximum of 90 proglottids rather than more than 200; the number of testes is 100 to 135 rather than 45 to 60; and the testes may extend laterally past the excretory vessels which never occurs in *C. praecoquis.*

The following cestodes have been reported from pocket gophers (*Geomys* and *Thomomys*).

Geomys breviceps Baird, Louisiana pocket gopher.
- *Monoecocestus anoplocephaloides* (Douthitt, 1915) n. comb.
  - Syn. *Schizotaenia anoplocephaloides* Douthitt, 1915.

Geomys bursarius (Shaw), Mississippi valley pocket gopher.
- *Andrya macrocephala* Douthitt, 1915
  - Syn. *Andrya microti* Hansen, 1947
    - *Andrya ondatrae* Rausch, 1948
    - *Andrya translucida* Douthitt, 1915
  - (?) *Andrya caucasia* Kirschenblatt, 1938
- *Cittotaenia praecoquis* (Stiles, 1895) Stiles and Hassall, 1896
- *Paranoplocephala infrequens* (Douthitt, 1915) Baer, 1927
  - Syn. *Anoplocephala infrequens* Douthitt, 1915
    - *Anoplocephala variabilis* Douthitt, 1915
    - *Anoplocephaloides infrequens* (Douthitt, 1915) Baer, 1924
    - *Anoplocephaloides variabilis* (Douthitt, 1915) Baer, 1924

Thomomys bottae bottae (Eydoux and Gervais), California pocket gopher.
- *Catenotaenia linsdalei* McIntosh, 1941

Thomomys talpoides clusius (Coues), Coues pocket gopher.

Explanation of Plate

All figures are of *Cittotaenia megasacca.* They were all made with the aid of a camera lucida, minor details and corrections being supplied freehandedly. Abbreviations: *cs,* cirrus sac; *d.ex.d.,* dorsal excretory duct; *esv,* external seminal vesicle; *ga,* genital atrium; *isv,* internal seminal vesicle; *ov,* ovary; *sr,* seminal receptacle; *t,* testes; *ut,* uterus; *v,* vagina; *v.ex.d.,* ventral excretory duct; *vd,* vas deferens; *vt,* vitellarium.

Fig. 1. Scolex.

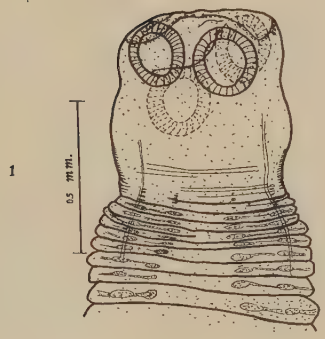
Fig. 2. Twenty-third to twenty-sixth proglottids. Dorsal view of young maturing proglottids. Composite drawings of frontal sections.

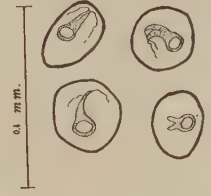
Fig. 3. Thirty-sixth to thirty-ninth proglottids. Dorsal view of fully matured proglottids. Only certain organs are shown in any one proglottid. The most anterior proglottid shows the testes which are dorsal in position. The next proglottid shows the cirrus sacs which are situated ventral to the testes. The third proglottid shows the seminal receptacle and vagina which are still more ventral. The fourth proglottid shows the ovary, vitellaria, and uterus which are ventrally situated. A superimposition of all four drawings would represent a single complete mature proglottid.

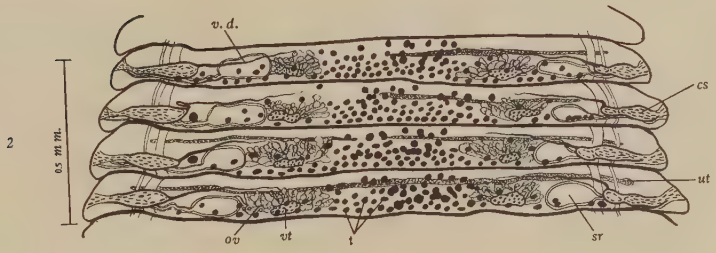
Fig. 4. Terminal (72nd.) proglottid. Dorsal view, frontal section.

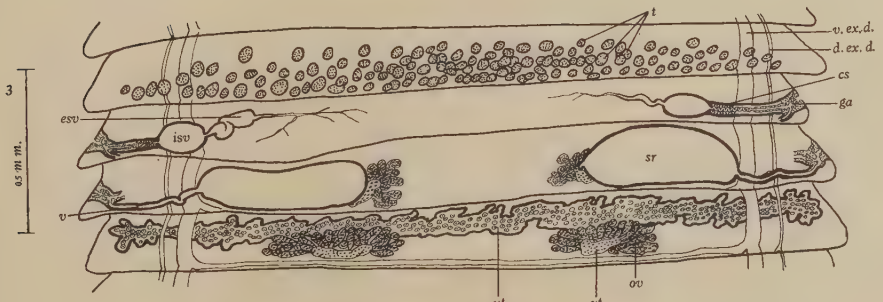
Fig. 5. Eggs drawn from frontal sections of the uterus.

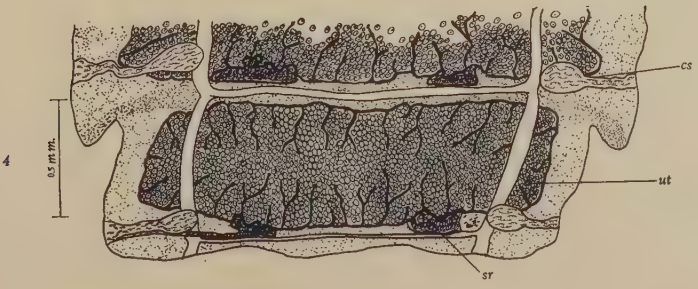
PLATE I


1
0.5 m.m.


5
0.1 m.m.


2
0.5 m.m.
v. d.
cs
ut
ov
vt
t
sr


3
0.5 m.m.
t
v. ex. d.
d. ex. d.
cs
ga
esv
isv
sr
v
ut
vt
ov


4
0.5 m.m.
cs
ut
sr

Cittotaenia megasacca n. sp.

Cittotaenia praecoquis (Stiles, 1895) Stiles and Hassall, 1896

Thomomys talpoides tenellus Goldman, Yellowstone Park pocket gopher.

Andrya macrocephala Douthitt, 1915

As noted by Freeman (1949), Fuhrmann (1931) pointed out that the generic name *Schizotaenia* Janicki, 1904 (Cestoda), was preoccupied by *Schizotaenia* Cook, 1895 (Myriapoda). Fuhrmann reinstated the generic name *Monoecocestus* Beddard, 1914. The name of the type species (*Taenia descrescens* Diesing, 1856), however, as noted by Hughes (1941), was a homonym of *Taenia descrescens* Rudolphi (in Creplin, 1849). Hughes proposed the new name *S. diesingi*, but Freeman points out that the name *Schizotaenia hagmanni* Janicki, 1904 was discovered by Baer (1927) to be a synonym of *S. decrescens*, and is available as a specific name. Apparently, the correct name of the type species is *Monoecocestus hagmanni* (Janicki, 1904) Freeman, 1941.

Freeman did not actually make all the additional new combinations implied in the synonymy of *Schizotaenia* to *Monoecocestus;* for example, *M. anoplocephaloides* (Douthitt, 1915), (see above), and *Monoecocestus sigmodontis* (Chandler and Suttles, 1933) Melvin and Chandler, 1950, synonym *Schizotaenia sigmodontis* from the cotton rat (*Sigmodon hispidus*).

SUMMARY

1. A new anoplocephalid cestode, *Cittotaenia megasacca,* from the pocket gopher, *Thomomys talpoides clusius,* is described and *Cittotaenia praecoquis* is reported for the first time from this gopher.

2. Cestodes reported from the pocket gophers, both *Geomys* and *Thomomys,* are listed with notes concerning the genus *Monoecocestus.* One new combination, *M. anoplocephaloides,* is made.

References

Arnold, J. G., Jr. 1938 A study of the anoplocephaline cestodes of North American rabbits. Zoologica, N. Y. Zool. Soc. **23:** 31–53.

Baer, J. G. 1927 Monographie des cestodes de la famille des Anoplocephalidae. Bull. Biol. France et Belgique. Supp. **10:** 1–241.

Douthitt, H. 1915 Studies on the cestode family Anoplocephalidae. Illinois Biol. Monogr. **1:** 351–446.

Freeman, R. S. 1949 Notes on the morphology and life cycle of the genus *Monoecocestus* Beddard, 1914 (Cestoda: Anoplocephalidae) from the porcupine. J. Parasit. **35:** 605–612.

Fuhrmann, O. 1931 Handbuch der Zoologie, Kükenthal-Krumbach. Cestoidea. 2(8) : 257–416.

———1932 Les Ténias des Oiseaux. Mém. Univ. Neuchâtel. **8:** 1–381.

Hansen, M. F. 1950 A new dilepidid tapeworm and notes on other tapeworms of rodents. Am. Mid. Nat. **43:** 471–479.

Hughes, R. C. 1941 The Taeniae of Yesterday. Bull. Okla. A. & M. Coll. **38** (16) : 1–83.

Joyeux, C. and Baer, J. G. 1936 Cestodes. Faune de France. Paris. (30) pp. 1–613.

McIntosh, A. 1941 A new dilepidid cestode *Catenotaenia linsdalei* from a pocket gopher in California. Proc. Helm. Soc. Wash. **8:** 60–62.

Melvin, D. M. and Chandler, A. C. 1950 New helminth records from the cotton rat, *Sigmodon hispidus,* including a new species, *Strongyloides sigmodontis.* J. Parasit. **36:** 505–510.

Rausch, R. and Schiller, E. L. 1949 A critical study of North American cestodes of the genus *Andrya* with special reference to *A. macrocephala* Douthitt, 1915 (Cestoda: Anoplocephalidae). J. Parasit. **35:** 306–311.

Stiles, C. W. 1897 A revision of the adult tapeworms of hares and rabbits. Proc. U. S. Nat. Mus. **19** (1105) : 145–237.

RESEARCH NOTES

A NOTE ON THE ECTOPARASITES OF THE JAVELINA, OR WILD PIG, *TAYASSU ANGULATUS* (COPE)

During the past five years, twenty specimens of *Tayassu angulatus* have been examined for ectoparasites, immediately after they were shot. These animals were taken in the following Texas counties: Aransas, Kleberg, Nueces, Terrell and Uvalde. Our chief interest has been to replenish our supply of the flea, *Juxtapulex porcinus*. Each javelina examined has shown at least a light infestation of this unusual flea. We have regarded it as absolutely host specific, since until recently no specimens have been seen on the numerous rodents and carnivores taken from the range of the javelina. Two female *J. porcinus* removed from a bobcat, *Lynx rufus,* in Uvalde County Texas, March 29, 1950, by O. L. Walker and C. W. Johnson, represent our first host record other than the wild pig.

Six species of ticks have been taken from the javelina: *Amblyomma americanum, A. cajennense, A. inornatum, A. maculatum, Dermacentor variabilis* and *Ixodes scapularis.* One animal examined was infested by the giant sucking louse, *Pecaroecus javalli.*—Richard B. Eads *Bureau of Laboratories, Texas State Department of Health.*

SPARGANA FROM THE FLORIDA ALLIGATOR

The author (Livro Jubilar Prof. Travassos, Rio de Janeiro, p. 337–341; 1938) described a *Diphyllobothrium* of the *mansoni* type (as contrasted to *mansonoides*) reared in a cat from spargana found in Florida water snakes (*Natrix*). This finding was proof that the true tropical and oriental form of *mansoni* occurs here in addition to the peculiar well-characterized native form, *D. mansonoides.* The present note is to establish the fact of a similar *mansoni*-like form proceeding from the alligator. On Nov. 16, 1949, 15 spargana were accidentally uncovered while dissecting two four-foot Florida alligators, from Manatee Co. Four worms were found in the muscles of one alligator, 11 in the other. These were divided and fed to 2 cats and a dog, known to be negative for *Diphyllobothrium.* The pressure of other work prevented examining the animals until Dec. 9, when one cat was found completely negative. The dog showed a few ova in the feces, but was negative on post mortem examination except for 3 small *Diphyllobothrium* found in its gut, having immature segments only, total length 6 to 7 cm. Evidently a mature worm had passed out spontaneously during the past few days, leaving only a few residual eggs. The third animal (cat), passing eggs at the time, was killed and found to harbor a large fully developed worm over 1 meter in length in the relaxed condition. This was fixed and preserved. The widest segments are about 6 mm. wide and about 5 mm. long. The posteriormost segments are over 1 cm. long and only 3 to 4 mm. wide. In the anterior mid-part of the chain the segments are about 5 mm. wide by 2.5 mm. long, and this proportion is maintained in the anterior region. The head and neck together, to the first evidence of segmentation, are 15 mm. long. The head itself is 3 mm. long. The bothria are somewhat wider in their anterior third, but at their widest are only a little more than 0.5 mm. while the widest part of the neck is only about 1 mm. The bothria are more flaring than in the illustration for the above article (Mueller, 1938).

The uterus, in the posterior, elongate, original segments of the worm is more drawn out and shows a posterior compact group of small caliber loops containing thin-shelled eggs, whose contents stain, and an anterior group of bulky loops containing heavy-shelled, stain-resistant yellow eggs. The ovary has the characteristic butterfly form. The cirrus and vaginal openings have the arrangement usual for the group, the vagina opening into a transverse pocket posterior to the cirrus, and the cirrus sac having the compound structure seen in *mansonoides* (J. Parasit. 23: 308–10; 1937). In the mid region of the strobila the genitalia are compressed antero-posteriorly, in accord with the changed shape of the proglottid, as has been shown by the author for *D. mansonoides* (J. Parasit. 21: 114–121; 1935). The piles of the outer section of the uterus, and the terminal chamber, are in all cases of the *mansoni* type. A careful study of the ootype region shows certain qualitative differences, but nothing that can be used to separate this worm from the many which have already been grouped together as *"D. mansoni."* The main difference between this worm and that previously raised from Florida water snakes is size. The present worm is 3 times as wide and 6 times as long as those previously raised from snake spargana. It is as large a *mansoni* as any on record, and certainly the largest recovered in this country.

The host relationship exhibited by the worm is suggestive. In the previous experiment in which spargana from Florida snakes were fed out, it was found in cats killed at the end of 2 weeks that both *mansoni* and *mansonoides* were present, but in cats killed at the end of 3 weeks

only *mansonoides* were present. This was taken as evidence that the cat is not the normal host. Apparently the same conclusion must be drawn from the present feeding experiments, in that of 15 spargana fed, only one mature worm remained at the end of 3 weeks. The presence of immature anterior sections and ova in the dog, would indicate that the worm may occasionally manage to develop to maturity, but cannot maintain itself in the dog or cat. Thus the true definitive host is probably one of the wild carnivores of Florida. Apparently no spargana of the *mansonoides* type were present in the alligator, since this species sustains itself indefinitely in the cat, and presumably in the dog also.

This case establishes conclusively the presence of two separate species of the *mansoni* group in the United States, and is a first record for spargana occurring in the alligator. One other point has been cleared up in the past ten years since the original work was done on these forms. At that time spargana had been found as a natural infection in water snakes, but it was felt that these animals could not normally serve to pass the worm along, since it was assumed that cats would not willingly feed on snakes. It was assumed that mice and other small mammals must be the natural intermediate hosts, since these were readily infected in the laboratory, even though extensive trapping failed to uncover infected mice etc. in the field. During the past decade, however, it has been determined that the meat of water snakes, at least, is relished by cats, presumably for its fishy flavor, a fact first determined when cats were discovered raiding a garbage can containing discarded snake carcasses. Thus there is no longer any doubt that the cycle in this country involves *Cyclops,* water snakes, and cats or other carnivora, and that other animals play only a minor or accidental role as intermediate host.—Justus F. Mueller, *College of Medicine, Syracuse University, Syracuse, N. Y.*

A REPORT OF *ECHINOCOCCUS GRANULOSUS* IN A HORSE IN THE STATE OF WASHINGTON

A registered Thoroughbred mare, aged about eight years, which was destroyed for examination following persistent sterility, showed two cysts in the liver at necropsy. The animal, which had been imported from England in 1947, had been kept at various places in western Washington (Centralia, Lake Sammamish, Skamokawa) for the ensuing two years. In April 1949 she was brought to Pullman, Washington, and kept there until destroyed on December 8, 1949.

According to Morgan and Hawkins (Veterinary Helminthology, Burgess Publishing Co., Minneapolis, Minnesota, 1924, 1949), there has been no report of *Echinococcus granulosus* (Batsch, 1786; Rudolphi, 1801) in domestic animals in the state of Washington. Cowan (1948, Jour. Wild Life Management, 12: 105–106), found *Echinococcus* in a Columbian Black-tail deer on Vancouver Island. Apparently this parasite is quite rare in the horse in the United States since a review of recent check lists failed to show the occurrence of this parasite in this host in North America. Megitt (The Cestodes of Mammals, London, 1924), without making any geographical reference, mentioned its presence in the horse and related species.

Each cyst was about 15 mm in diameter and had a tough, whitish, connective tissue capsule less than 1 mm thick. The inner surface showed numerous, tiny, yellowish-white, granular structures. Wet mounts prepared from scrapings of the cyst lining showed numerous invaginated and evaginated scolices bearing suckers and a double crown of hooks. Sections of the cyst, when stained with Ehrlich's acid hematoxylin and eosin, showed a connective tissue capsule, laminated cuticle, germinative layer and occasional brood capsules containing invaginated scolices. Surrounding liver tissue showed compression and was infiltrated with eosinophils in areas adjacent to blood vessels. Measurements taken from the cyst wall would suggest that the cyst was in an early stage of development.—Donald R. Cordy and Margaret Gardiner Eastlick, *Department of Veterinary Hygiene and Pathology, Washington State College, Pullman, Wash.*

GROUP TREATMENT WITH CARICIDE FOR ASCARIASIS IN POULTRY

Caricide was shown by Hewitt, *et al.* (1947, J. Lab. and Clin. Med., 32: 1314–1329; and ibid., 32: 1304–1313) to have a remarkable effect against *Litomosoides* infections in rats. A similar influence of Caricide has been shown against *Dirofilaria immitis* in dogs (Hewitt, *et al.,* 1947, J. Parasitol., 34: 237–239), *Wuchereria bancrofti* in man (Santiago-Stevenson, *et al.,* 1947, J. Am. Med. Assoc., 135: 708–712), adult *Trichinella spiralis* in rats (Oliver-Gonzales and Hewitt, 1947, Proc. Soc. Exp. Biol. and Med., 66: 254–255), and ascariasis in dogs (Kanegis, 1948, J. Am. Vet. Med. Assoc., 113: 579–589; and Oliver-Gonzales and Hewitt, 1947, Proc. Soc. Exp. Biol. and Med., 66: 254–255).

Riedel (1950, Poultry Sci., in press) investigated the anthelmintic value of Caricide in chickens infected with *Ascaridia galli.* The best results (69.4 percent effectivity) were obtained by an oral dose of 1.0 gm. followed several hours later by a 0.5 gm. redose. In view of the poor

results obtained by individual treatment, it was decided to note the anthelmintic effect of Caricide upon *A. galli* in chickens when administered in the feed.

The Caricide incorporated in the feed at various levels of concentration was administered to 8-week old New Hampshire birds heavily infected with mature *Ascaridia.* The weight records before and after treatment; and the worms passed during the treatment period, and those still harbored at the end of the treatment period were used to determine the anthelmintic efficiency of the drug. Four groups of 10 chickens each were treated at each of the dosage levels.

The weight records showed that the Caricide incorporated in the feed at 0.25, 0.5, 1.0, and 2.0 percent concentrations and administered for 1 week to chickens was not toxic. A feed containing 5.0 percent of Caricide was toxic and unpalatable.

The chickens fed a ration containing 0.25 percent Caricide eliminated an over-all total of 12.0 percent of the *Ascaridia* harbored; those given a 0.5 percent feed eliminated 28.6 percent of their ascarids. A 1.0 percent feed was 50.0 percent effective, while a 2.0 percent feed was 81.0 percent effective. The 5.0 percent diet caused no elimination because of its unpalatability. None of the control birds passed worms during the experimental period.

In another test 30 heavily infected chickens fed 1.0 percent of Caricide for a 2-week period and purged with epsom salt at the end of the first and second weeks eliminated 89.2 percent of their ascarids; 30 similarly-treated, unpurged birds had 72.0 percent elimination; while 15 untreated, unpurged controls failed to pass ascarids.—Bernard B. Riedel. *Disease Research, Poultry Department, University of Georgia, Athens, Georgia.*

THE USE OF ANTIBIOTICS IN ARTIFICIAL MEDIA FOR IN VITRO EXPERIMENTS WITH ACANTHOCEPHALA

The chief difficulty encountered in studies on the physiology of intestinal helminths is contamination of the medium in which they are kept. In this respect the Acanthocephala seem to present more difficulty than other intestinal parasites; a heavy growth of micro-organisms develops within a few hours and some of the worms usually die in twenty-four hours. In experiments with *Macracanthorhynchus hirudinaceus,* the thorny-headed worm of hogs, an effort was made to reduce contamination by using chemically clean glassware, washing the worms several times in the medium before beginning the experiment, placing the worms individually in small flasks each containing about 100 ml. of a modified Ringer-Tyrode solution, and changing the medium every eight hours. With this technique, about 80% of the worms survived in an incubator at 38° C. for 72 hours, as evidenced by the fact that they responded to mechanical stimulation. However, none of the worms exhibited spontaneous movements after the first 24 hours of the experiment. Few of the worms lived more than 72 hours. With the ordinary technique, most of them appeared to be dead in less than 48 hours.

Since a combination of streptomycin and penicillin was effective in purification of hemoflagellate cultures (Seneca *et al.,* Amer. J. Trop. Med. **29**: 41; 1949), an experiment was conducted with *M. hirudinaceus* using the Ringer-Tyrode solution to which these antibiotics had been added in the proportions of 50 mg. of dihydrostreptomycin sulfate and 140 mg. of penicillin (crystalline G) to 1 liter of medium. The previous technique was used, except that the medium was not changed during the course of the experiment. Ten adult female worms were placed individually in Erlenmeyer flasks and observed for a period of six days. During this time the medium remained clear in all of the flasks. At the end of three days spontaneous movements were observed in all of the worms. By the end of six days two worms had died and the remaining eight no longer exhibited spontaneous movements, although they did respond to mechanical stimulation. These results suggest that a combination of streptomycin and penicillin might prove successful in controlling the growth of micro-organisms in media in which intestinal helminths are to be kept.—Helen L. Ward, *University of Tennessee, Knoxville, Tennessee.*

EXOERYTHROCYTIC STAGES IN *PLASMODIUM HEXAMERIUM*

Although exoerythrocytic stages have now been demonstrated in a number of species of plasmodia, including malarial parasites of birds, reptiles, monkeys and man, they have so far been sought without success in the avian plasmodia often referred to as the group comprising the "smaller species." Included in this group are *Plasmodium hexamerium, P. nucleophilum, P. vaughani, P. rouxi,* and probably *P. polare.* All of these species produce elongate gametocytes, are relatively small in size, produce small numbers of merozoites per segmenter, and fail to displace the host cell nucleus. The infections they produce are very chronic and similar in type. All in all, they seem to make up a natural biological group.

For this reason, the finding of exoerythrocytic stages ("phanerozoites" ?) in the brain of an orange-crowned warbler infected with *Plasmodium hexamerium* is of considerable interest, since it suggests that with sufficient search such stages may also be found in the life-cycles of the other

members of the group. The preparations in which the parasites were found were J.S.B.-stained spreads or smears. Although few in number (only 12 E.E. stages could be found in a smear with an area of 4 cm.^{2} despite meticulous examination with the 5x ocular and oil immersion objective), the appearance of the forms was entirely typical. The only differences noted were the lack of any vacuoles, and the relatively small size. One apparently mature segmenter was seen, and it had about 35 merozites. The parasites were contained in endothelial cells of the capillaries, as in other plasmodial species. No E.E. forms could be found in smears of the lungs and spleen.

The infection in the warbler was of course a mosquito-induced one, but no E.E. stages have been found in a number of song sparrows and a cowbird, nor in blood-induced infections in canaries. The warbler had no other blood parasites, and there is therefore little question that the forms seen were E.E. stages of *P. hexamerium.* It was trapped in Syracuse.—REGINALD D. MANWELL, *Department of Zoology, Syracuse University.*

A METHOD OF PRESERVING UNSTAINED THICK FILM

Wilcox (1943; Nat. Institute Health Bulletin No. 180, Washington, D. C.) suggested that unstained thick film may be successfully kept in a cool place provided care is taken to prevent condensation of moisture on the slides. No mention, however, was made in regard to the length of time of preservation and the method used to accomplish this end. It seems timely, therefore, to describe a method to fulfill such need and to evaluate it on the basis of our observations.

Thick films are prepared from infected blood, with or without the use of anticoagulant, dried as in routine making of thick films and placed in a wooden box with a capacity of 25 slides. After sealing the lid and seams with Scotch tape, the box is wrapped with at least 2 or 3 large sheets of cellophane and tightly sealed. This package is then wrapped with hard paper, preferably of water repellent quality, securely sealed and stored in a refrigerator at 5° C. The slides were actually placed in the refrigerator within eight hours after being made. Three weeks following the storage of the slides in this manner, any moisture which has condensed on the slides during storage is removed at room temperature as in the original preparations. They are then stained with the diluted Giemsa solution according to the method of Barber and Komp (1929; Pub. Health Rep. 44: 2330).

It was found that the malarial parasites retained their characteristic morphology and staining properties comparable to those seen in freshly made thick films. The chromatin granules appeared red to purplish red, while the cytoplasm colored blue, with pigment granules in the older forms. Leucocytes, blood platelets and reticula of young red cells likewise exhibited the characteristic appearances observed in freshly prepared films. Moreover, the loss of parasites during the storage period of three weeks was surprisingly small. Fifteen counts each were made on the thick films at the beginning and at the end of the storage period, and the average count of each determined. At the beginning there were 31,611 parasites per c. mm., while at the end of the storage period the count was 27,998 per c. mm., indicating that the loss of parasites during the process was negligible.

It is suggested, therefore, that the method may serve as a valuable aid in instruction in cases where fresh source of the material is not readily available.—H. TSUCHIYA AND JOSEPH E. MCCOY, *Washington University School of Medicine, St. Louis, Mo.*

HELMINTHS FROM FISHER *(MARTES P. PENNANTI)* IN MAINE

During the 1950 trapping season (January) 36 fisher *(Martes pennanti pennanti),* largely from northern Maine, were examined for helminths. The skinned carcasses were obtained from trappers in cooperation with the Maine Cooperative Wildlife Research Unit. The helminth fauna of the fisher is inadequately known, as only two papers, each involving a single but different species of nematode, have been encountered. Chitwood (1932. J. Parasit. 18: 307) reported *Uncinaria stenocephala* from fisher, *Mustela* sp. [sic] taken in Canada, and Morgan (1943. *Ibid.* 29: 158–159) reported *Soboliphyme baturini* from *Martes p. pennanti* (host locality not given). Since our findings include species previously unreported from the fisher, they seem worthy of record.

Of the 36 animals autopsied, 27 were positive for one or two species of helminth. In 21, the infection was limited to one species; in six, double infection occurred. Eighteen were positive for *Mesocestoides variabilis* Mueller, 1927; nine positive for *Ascaris mustelarum* Rudolph, 1819 (= *Ascaris martis* Gmelin, 1790; Non Schrank, 1788); and *Crenosoma* sp. was found in one host. Since only a single female of the latter was available, specific determination was not possible. Double infections involved *M. variabilis* and *A. mustelarum,* and *A. mustelarum* and *Crenosoma* sp.

Grateful acknowledgment is due Mr. Malcolm W. Coulter, Assistant Leader of the Maine Unit, for his cooperation in making the hosts available and Dr. Asa C. Chandler, The Rice In-

stitute, for kindly verifying the identification of *Mesocestoides variabilis*.—MARVIN C. MEYER, *Department of Zoology, University of Maine, Orono, Maine* and B. G. CHITWOOD, *Department of Biology, The Catholic University of America, Washington, D. C.*

MENTHOL RELAXATION OF HELMINTHS BEFORE FIXATION

Parasitologists sometimes have difficulty in obtaining specimens, especially of flukes, cestodes and acanthocephalans, in relaxed condition when fixed in preparation for whole mounts or histological sections. The following are some of the more common methods used with varying degrees of success: 1) immersing the material suddenly in a hot fixative; 2) shaking in saline solution with or without mercuric chloride, according to various modifications of the method of Looss (Zool. Anz. 24, 1901); 3) freezing the host and consequently killing the parasites in a relaxed condition; 4) pressing the parasite between glass slides or under a cover slip, the pressure being maintained to prevent it from contracting while being fixed.

Various anesthetics, among them menthol, have been used for relaxation of Protozoa, small invertebrates, and larval forms (A. E. Galigher, 1934, Essentials of Practical Microtechnique, pp. 60–61). However, parasitologists have not commonly used anesthetics or narcotics for relaxing parasites. The writer used menthol very successfully to get complete relaxation of snails for anatomical studies. In the course of this work, it was observed that cercariae were also relaxed so that they could easily be studied. At the suggestion of Dr. Asa C. Chandler at the Biological Station of the University of Minnesota, where this study was made, menthol anesthetization of various helminths was tried. The helminths were placed in stender or petri dishes containing tap water or dilute saline solution at room temperature, and menthol in powdered or crystalline form was sprinkled on the surface of the water in an excess of the amount needed for a saturated solution. Half a gram of menthol added to 100 cc. of water is sufficient to cause relaxation of the parasites, although only a very small amount dissolves. The time necessary for complete relaxation depends on individual specimens and on whether saline or tap water is used; a longer time is required for relaxation in saline than in water. The solubility of menthol in water increases with temperature; thus a parasite relaxes more easily if the temperature of the water is slightly above room temperature. The parasite should be tested with a very fine brush to determine the loss of irritability. The specimen may then be fixed in any of the usual fixatives without danger of contraction.

This method was employed with satisfactory results with the following: adult flukes, metacercariae, cercariae, rediae, cestodes, acanthocephalans, nematodes, and leeches. In the case of nematodes there is a slight advantage over fixation in hot formalin or 70% alc., and for cestodes, soaking in plain tap water as advocated by Chandler is just as good, though with menthol, relaxation occurs in a shorter time. For the other groups of parasites, however, the method has distinct advantages over the methods usually employed. In the case of cestodes best results are obtained by spreading on a glass plate, and applying the fixative along the sides with a medicine dropper. After allowing action for a few minutes, the parasites are immersed in the fixative to complete fixation. Elongated Acanthocephala and leeches are also advantageously treated in this manner. There is no difficulty in getting full extension of such organs as the proboscis of Acanthocephala, the rostellum of cestode scolices, and the earlike processes of some species of *Alaria*.

Since menthol is sparingly soluble in water (.04 in 100 parts at room temperature) but very soluble in alcohol an alcoholic solution proved to be very convenient in this method. The following gave satisfactory results with some adult flukes, cercariae, tape worms and acanthocephalans: about 24 gms. of menthol are dissolved into 10 cc. of 95% alc.; one drop of this saturated solution added to 100 cc. of water is enough to saturate the water with menthol and acts faster in relaxing the parasites than when menthol crystals are sprinkled on the surface of the water. The alcoholic solution of menthol can be kept for a long time for further use. In flukes containing a large number of eggs expulsion of the eggs should be accomplished first by leaving them in water for from 1 to several hours before applying the menthol solution to them. The writer wishes to acknowledge the help and interest of Dr. Asa C. Chandler and of Miss Jean DeBell of the Department of Zoology, University of Minnesota.—EMILE T. ABDEL-MALEK, *Department of Zoology, University of Michigan.*

CERCARIA SEGMENTINAE TANABE 1948, A HOMONYM OF *CERCARIA STURNIAE*

Tanabe (1948, J. Yonago Med. Assoc. 1: 2–3) briefly described a new species of furcocercous cercaria as *Cercaria segmentinae* n. sp. This name was subsequently discovered to be a homonym of *Cercaria segmentinae* Faust 1926. Consequently, it becomes necessary to rename *Cercaria segmentinae* Tanabe 1948, and it is now redesignated as *Cercaria sturniae* n. sp. Subsequent experimental and field work indicates that the adult has characteristics placing it in the genus *Gigantobilharzia* Odhner 1910. It is a long thread-like worm with an anteriorly placed genital

pore and acetabulum, the latter being very indistinct or possibly absent. *Cercaria sturniae* now should be designated *Gigantobilharzia sturniae* (Tanabe 1948) n. comb.—H. Tanabe, *Department of Pathology, Okayama Medical School, Okayama, Honshu, Japan.*

METHYL TESTOSTERONE IN THE DIET OF CHICKS AND GROWTH OF THE NEMATODE *ASCARIDIA GALLI*[1,2]

Sadun (J. Parasit. 34 (Suppl.) : 18, 1948) reported a relation of the gonadal hormones to the growth of a nematode, *Ascaridia galli,* in White Leghorn chicks. Following experimental exposure, his control chicks retained 2.02 to 4.02 times as many worms as his treated birds which had received repeated "moderate" injections of testosterone propionate. Treated birds which received "very heavy" doses of testosterone, however, harbored 3.95 times as many worms as their untreated controls. Injections of testosterone were found to cause an initial increase and a later retardation of growth of the worms.

In preliminary studies on the relation of gonadal hormones to the development of *A. galli* in New Hampshire chicks at the Kentucky Agricultural Experiment Station, methyl testosterone was fed at the rate of 20 mg/kg growing mash. Treated chicks, exposed to 50± infective *A. galli* eggs, were found to harbor the same number of worms as their untreated controls at postmortem. In the 3-week test period following exposure, the treated birds gained an average of 78.2 grams less weight than their controls, although this difference has no statistical significance perhaps because of the small number (ten) of birds tested. Worms recovered from the treated birds ranged in length from 14.33 to 28.33 mm. and averaged 23.43 mm.; 3.5 worms were present, on average at postmortem. Worms recovered from the untreated controls ranged in length from 2.83 to 21.50 mm., averaged 13.80 mm., and 3.5 worms were present, on average, at postmortem. The difference in mean worm length yielded an F ratio which exceeded the 1 percent level in an analysis of variance and is, therefore, highly significant.—A. C. Todd and D. H. Crowdus *Ky. Agric. Expt. Sta., Lexington, Ky.*

ACUTE MALARIA IN A CANADA JAY OF THE HIGH ROCKIES

The number of bird species known to harbor malarial plasodia has continued to grow ever since Danielewski, 65 years ago, first saw pigmented parasites in avian blood. Although the list of susceptible species now numbers several hundred, the finding of new hosts is still of interest. For this reason, and because local environmental conditions would seem to make the occurrence of any type of malarial infection unlikely, the case below is significant. On July 24, 1950, a Canada Jay *(Perisorcus canadensis canadensis)* was brought to the Rocky Mountain Biological Laboratory, Crested Butte, Colorado, for examination, after being trapped. A blood smear showed a heavy infection with *Plasmodium cathemerium,* and autopsy revealed a very large, dark colored spleen. Smears of the brain and lungs were also examined, but no E.E. forms were seen, perhaps because the infection had been too recently acquired. It is probable that birds of many species more commonly pick up their malarial infections in the southern portion of their ranges, but since the Canada Jay is not a migratory bird it must have become infected locally. That malarial plasmodia can develop in mosquitoes at this altitude (almost 10,000 feet), where temperatures at noon seldom exceed 80° F., and at night often fall to freezing or even below, is rather remarkable, especially since the summer season lasts only about six weeks. That avian malaria is indeed rather uncommon in this area is suggested by the fact that none of 46 cliff swallows, 6 broad-tailed humming birds, and 2 white-crowned sparrows were found infected (though these are migratory species). Two other Canada Jays were also malaria-free.—R. D. Manwell, *Syracuse University, Syracuse, N. Y.*

ADDITIONAL NOTES ON THE HELMINTH PARASITES OF THE BOB-WHITE IN TEXAS

Previous parasitic examinations of Bob-white Quail *(Colinus virginianus)* from Texas have been reported by Webster (1944, Trans. Amer. Micr. Soc., 63: 44–45; 1948, Trans. Amer. Micr. Soc., 66: 339–343; 1948a, J. Parasit., 34: 84–86) and Webster and Addis (1945, J. Parasit., 31: 286–287). From northwestern Denton County, in North Texas, nine quail were examined in November, 1946, but no worms were found. From Kleberg County, in South Texas, the small intestines, only, of 38 additional quail, taken in December, 1946, were examined. Three

[1] The investigation here reported is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] The methyl testosterone used in these studies was furnished by The Upjohn Company, Kalamazoo, Michigan.

were infested with unidentifiable cestodes, three with *Raillietina (Raillietina) klebergi* Webster, 1948, and one with *Raillietina (Paronniella) minuta* Webster, 1948.

When the original description of *Raillietina minuta* was written (Webster, 1948; *op. cit.*) no scolex was available. The single specimen found in the present collection has a complete scolex which may be described as follows: scolex 223 μ wide, bearing ellipsoid suckers 59 to 76 μ in longitudinal diameter and armed with four rows of spines 6 to 8 μ long. Retracted rostellum 51 μ in diameter, bearing 84 hooks, each 13 μ long, and arranged in a double row. Posterior row of hooks set back from the anterior row about 1 μ. Aside from the scolex, this specimen differs from the original description on two minor points. The cirrus pouch attains a greater maximum diameter—to 37 μ—and the extruded cirrus is longer—59 and 70 μ, respectively, in two segments—than in any of the original material.—J. Dan Webster, *Hanover College, Hanover, Indiana.*

A TECHNIQUE FOR THE RAPID PREPARATION OF TAPEWORMS FOR IDENTIFICATION

When the rapid identification of large numbers of tapeworms is necessary the following technique has proved to be reasonably satisfactory. The worms are relaxed in tap-water and then, as recommended by Meggitt (1924; Parasitology **16**: 266–268), they are placed in lacto-phenol (one part 85% lactic acid, one part phenol, one part glycerine and two parts water) which simultaneously fixes, clears and preserves the worms. Temporary whole-mounts in lacto-phenol (which have been found to be satisfactory, without ringing, for as long as thirty days) are then made and the worms are studied with the aid of phase difference microscopy, the 16 mm. and 4 mm. medium dark contrast objectives (American Optical Company) proving to be particularly useful. The work was done under a grant from the Dr. Hess and Clark, Inc. and the direction of Dr. D. E. Cooperrider, the University of Georgia.—Frank H. Dowell, *the Department of Zoology and Entomology, the University of Tennessee, Knoxville.*

NOTES ON A SMALL COLLECTION OF BLOOD SMEARS OBTAINED FROM AMPHIBIANS AND REPTILES IN MARYLAND DURING THE SPRING OF 1950

(Carried out during the tenure of a temporary special fellowship from the United States Public Health Service.)

Except where otherwise noted the material discussed in this contribution was collected at various points along the old Chesapeake and Ohio Canal from Glen Echo to the Great Falls of the Potomac, during the months of April and May.

Thin smears of heart blood were made in the majority of cases. In the case of the few turtles handled, blood was obtained by snipping off the tip of the tail. All preparations were air dried, and following my return to New Zealand they were fixed in absolute methyl alcohol and stained with Giemsa.

The material studied comprised smears from 20 amphibians belonging to 6 species, and 10 reptiles belonging to 3 species. Haematozoa were found in the blood of one of these animals only, a 61 mm. adult of *Rana palustris* Le Conte, the pickerel frog. This frog, collected at Glen Echo on May 11th, had a moderately heavy infection of a large, polymorphic trypanosome. Some 20 parasites were present in each of the 3 smears obtained.

The trypanosome concerned varies in shape from a relatively slender form tapering to a point at both extremities, and having a prominent undulating membrane and a short free flagellum, to a large, shapeless form with a very narrow undulating membrane and no free portion to the flagellum. In all cases the body is exceedingly thin, staining pale blue with Giemsa, and has many longitudinal myonemes. The smallest example seen measures 41.5 μ in length by 18.1 μ in breadth at the nucleus, while the largest measures 76.4 μ by 41.1 μ in its greatest dimensions. A few of the trypanosomes have an elongate nucleus measuring some 7.5 μ by 4.0 μ, but in most cases the nucleus is of circular shape and about 4.0 μ in diameter. This structure, which usually has a prominent karyosome, is posteriorly situated, and is located from 56.3% to 76.3% of the total body length from the anterior extremity. The parabasal body, which is located at the center of a circular non-staining area, lies close to the nucleus (8.0 μ to 10.0 μ distant from this structure) in the posterior part of the body. In the more typically trypanosomal parasites the well developed undulating membrane is thrown into a number of folds, and averages 3.0 μ in width.

From its morphology and measurements, the trypanosome of *Rana palustris* described above is identified as *Trypanosoma rotatorium* (Mayer). Nigrelli (1945; Zoologica 30: 47–56) examined many amphibians from the eastern and southern United States for Haematozoa, and gave a resume of the North American literature in this field. None of the earlier investigators had

reported trypanosomes from the blood of *Rana palustris*, and Nigrelli himself found this frog negative for Haematozoa. The host record is thus regarded as new.

A list of the amphibians and reptiles examined for Haematozoa with negative results during the present study is given below. Various investigators in other localities have reported blood parasites from all these species, although in many cases both the incidence and intensity of infection have been very low (e.g. in the Hylidae—Nigrelli, 1945):

Bufo americanus americanus Holbrook (1); *Hyla crucifer crucifer* Wied (13); *Hyla versicolor* (Le Conte) (1); *Plethodon cinereus cinereus* (Green) (3); *Triturus viridescens* (Rafinesque) (1); *Chrysemys picta picta* Schneider (1); *Sceloporus undulatus* (Latreille) (7); and *Terrapene carolina carolina* (Linnaeus) (2).—MARSHALL LAIRD, *Flight Lieutenant, Royal New Zealand Air Force, Department of Zoology, Victoria University College, Wellington, New Zealand.*

A NOTE ON *RIBEIROIA ONDATRAE* PRICE, 1931, (TREMATODA)

In October 1950, at Rockwood, Ontario, an investigation of a disease in a flock of domestic geese revealed moderately heavy infestations with *Echinostoma revolutum* (Fröhlich, 1802). Among these echinostomes was a solitary specimen of *Ribeiroia ondatrae* Price, 1931, (syn. *Psilostomum ondatrae*). The measurements of the specimen from the goose agree closely with those described by Price and Beaver for the species. Among previously known avian hosts of this parasite are gulls (*Larus californicus* and *L. argentatus*), osprey (*Pandion haliaetus carolinensis*), Cooper's hawk (*Accipiter cooperi*) and domestic chickens. Experimental infestations have been produced in ducks, pigeons and canaries.

No previous records of *R. ondatrae* from the domestic goose in Canada are known.

Acknowledgment for assistance in identifying this specimen and reviewing host lists is gratefully made to Dr. L. P. E. Choquette, Institute of Parasitology, Macdonald College, Quebec.—A. A. KINGSCOTE, *Department of Parasitology, Ontario Veterinary College, Guelph, Ontario, Canada.*

HYMENOLEPIS DIMINUTA IN THE SYRIAN HAMSTER

In view of the increasing use of the hamster as a laboratory experimental animal it seems worth while to report that this animal may be quite readily infected with the cestode, *Hymenolepis diminuta*. About a year ago Beck and Read (unpublished experiments) experimentally infected the hamster with a strain of this worm which had been maintained in albino rats at the Rice Institute for fourteen years. The writer has recently found natural infections in hamsters obtained from a commercial source in the Los Angeles area. The Los Angeles strain was quite easily established in albino rats. These observations indicate that there is little or no host specificity of different strains of this worm insofar as rats and hamsters are concerned.—CLARK P. READ, *University of California, Los Angeles.*

TRYPANOSOMA VESPERTILIONIS AND *LITOMOSOIDES SP.* IN *EPTESICUS FUSCUS FUSCUS*

Many investigators have reported finding *Trypanosoma vespertilionis* in bats from both North and South America. As far as the writer can determine, the known host range does not include *Eptesicus fuscus fuscus*. The known locales in North America from which *T. vespertilionis* has been reported are either in southwestern United States or California. The bats dealt with in this report were collected from caves in Alleghany county, Virginia. Whether this constitutes an autochthonous case is uncertain, since *Eptesicus f. fuscus* is known to range from the northern limit of tree growth to as far south as Panama.

Seven bats were examined, blood being taken by heart puncture. A preliminary microscopic examination of freshly drawn blood was made and later stained slides were examined. *T. vespertilionis* was found in four bats and microfilaria of *Litomosoides sp.* in two. In one bat both parasites were found. One of the bats harboring microfilaria was sacrificed and autopsy revealed a single adult female filaria in the body cavity. (The filaria was identified by Dr. B. G. Chitwood)

In view of the fact that some investigators believe *T. vespertilionis* and *T. cruzi* to be the same organism, the apparent high incidence in the common house bat may be of some significance. Further work is planned on larger numbers to test the frequency of occurrence in bats from this area.—FRANCIS G. TROMBA, *University of Maryland, Dept. of Zoology, College Park, Md.*

COLLEMBOLA AS FOOD FOR CHIGGERS (ACARINA· TROMBICULIDAE)

A number of insects and their eggs were offered as food to nymphal and adult chiggers at the University of Kansas during the spring and summer of 1948. Some spider eggs as well as

mites and their eggs also were used in chigger cultures. Of these foods, a large number of insect eggs (particularly those of Dermaptera, Diptera, Homoptera, and the smaller members of the Hemiptera and Lepidoptera) and some spider eggs (Agelinidae and Lycosidae) were found highly satisfactory for this purpose. Active stages of some mites may be utilized as food but there is little conclusive evidence that mites contribute extensively to the food of chiggers. Tyroglyphid eggs may have been a source of food to an undetermined species of *Walchia*, since this culture was maintained for over a nine month period in the presence of tyroglyphid mites. The nymphs and adults of the chiggers were not seen feeding during this period.

The insect eggs were obtained by ovarian dissection (using the entire ovaries) of gravid females collected by sweeping in the field. The use of dissected eggs of appropriate insects presented some difficulties; firstly, sanitary conditions in chigger cultures were difficult to maintain owing to large amounts of mold growth; secondly, it was constantly necessary to supply fresh ovarian material to cultures. Consequently, chigger cultures required many hours of attention. On the other hand, spider eggs were acceptable to chiggers only in the earliest stages of egg differentiation and the problem of collecting such eggs limited their general use. The principle limiting factor for field collected gravid female insects was their seasonal abundance and supply.

Coincident with the feeding to chiggers of dissected insect ovaries and eggs and spider eggs, several species of Collembola were cultured. These collembolan cultures were maintained on pieces of banana which were later replaced by active dried yeast pellets. Several species of collembolans were introduced into some chigger cultures with the hope their presence in cultures would result in more sanitary chigger culture conditions. Members of the Entomobryidae, Poduridae, and Sminthuridae soon were established as mixed cultures with chiggers. Several months of observation indicated their value in cultures, although the Sminthuridae were only of minor importance. The chiggers in these mixed cultures required less attention and there were fewer casualties from disease. Most species of molds were kept to a minimum. The entomobryids were significantly helpful in cleaning the undesirable debris and molds from the quiescent, transforming stages of chiggers.

It was during the development of satisfactory methods for culturing and successfully rearing nymphs and adults in the laboratory that a species of Entomobryidae, *Sinella curviseta* Brook, was found to lay eggs that were suitable as food to the nymphal and adult chiggers of some genera. The active Collembola themselves were preyed upon by other genera; whereas some preyed upon both the active and egg stages. Thus a colony of *S. curviseta* can be kept in the same culture dish with the chiggers, supplying food to the chiggers as needed. The Collembola require no food in addition to the active dried yeast pellets. Stock cultures of this collembolan have been maintained in the laboratory for two years with the food consisting of nothing but yeast. In chigger cultures small amounts of additional food could be obtained such as some molds, dead chiggers and debris.

Species other than *S. curviseta* in the Entomobryidae were not sufficiently prolific and were too small to be of much importance although their eggs were also eaten. The podurids supplied no food whatsoever, neither the eggs nor the active forms being eaten by the nymphs or adults of chiggers. Consequently, some podurids multiplied so rapidly that they soon developed a nuisance value. Poduridae which became well established in chigger cultures were difficult to eliminate and a transfer of chiggers into a new culture dish was the practical solution to the problem.

Sinella curviseta also can become sufficiently abundant to be objectionable and even detrimental in chigger cultures if permitted to multiply unchecked. Crushing collembolans in cultures is the general practice to keep their numbers at an optimum. The Collembola killed in this manner are readily eaten (if crushed sufficiently) by the surviving individuals. Also, this practice of wounding or crushing was employed generally in cultures containing species of chiggers which fed exclusively or in part upon the collembolans themselves, since these chiggers feed most readily on injured or molting Collembola rather than on active ones. Chiggers which feed upon such collembolans must constantly have a fresh supply of food, reducing the number of collembolans in these cultures; therefore, it often becomes necessary to add *Sinella* to them. It is not practical to withhold yeast from a culture to limit their numbers. If food is limited, the collembolans will feed upon the nymphal and adult chiggers, as a rule attacking their posterior ends and in severe cases eating half the chigger to the genital plate ventrally and beyond the plate dorsally. Also, to limit the food for collembolans will limit the laying of eggs. The elimination of collembolan eggs from cultures will often result in the chiggers eating their own eggs. An alternative for crushing collembolans in cultures is to invert the culture over a clean dish, jar them loose, and return any chigger that may fall out to its original culture dish.

Genera that have been successfully cultured in company with *Sinella* include *Acomatacarus, Euschöngastia, Hannemania, Neoschöngastia, Trombicula, Walchia* and two undescribed genera. In all about 25 species have been reared to the adult stage in the laboratory, some of the genera

listed above being represented by several species. Nymphs of a few additional species were obtained. Eggs and larvae were subsequently obtained from approximately 15 species reared to the adult stage.

In general, species of *Neoschöngastia,* two species of an undescribed genus, and several species of *Euschöngastia* and *Trombicula* fed exclusively upon the active forms of *S. curviseta.* Some species of *Euschöngastia* and *Trombicula* fed upon the active forms as well as the eggs of *S. curviseta.* All the species of *Acomatacarus, Hannemania, Trombicula* (*Eutrombicula* and *Neotrombicula*) and some species of *Euschöngastia* and *Trombicula* (undescribed subgenus) fed exclusively on eggs. The food of a species of *Walchia* was not determined.

The Holarctic species *Sinella curviseta* Brook was determined by Harlow B. Mills who states (in correspondence with C. D. Michener); "In North America most collections have come from greenhouses and similar protected environments, and it is possibly an introduced species. I have found, too, that it cultures very easily." It is quite possible that this species was obtained from the greenhouse which is part of the chigger laboratory at the University of Kansas. Stock cultures of *S. curviseta* are now maintained for distribution to other workers.

The studies upon which this paper is based were conducted under a contract, N6 ori-220, Task Order II, between the University of Kansas and the Office of Naval Research.—Louis J. Lipovsky, *Department of Entomology, University of Kansas, Lawrence, Kansas.*

ASCARIDIA GALLI IN HEN'S EGG

Recently a University of Idaho student brought a nematode to the author for identification. The specimen had been found hanging from the side of an infant's mouth while the child was having its morning feeding of egg and Pablum. The nematode was dead but still in good histological condition. It was immersed in 70% alcohol, then cleared rapidly in 90% phenol and examined. The specimen proved to be a sexually mature male, 46 mm. in length. Upon comparison it was found to be identical to males of *Ascaridia galli* from the author's collection. The egg had originally been cooked approximately 1–1½ minutes and then mixed with the Pablum. It is quite possible that in this case the ascarid had gained access to the egg while the latter was passing down the oviduct.—Stewart C. Schell, *Department of Biological Sciences, University of Idaho.*

HELMINTHS IN CATS FROM PANAMA CITY AND BALBOA, C. Z.

The present communication records the intestinal helminths on 96 cats captured in Panama City and Balboa (Canal Zone). Foster (1939, Helm. Soc. Wash., 6: 101) reported his findings in cats examined in Panama City. If we consider the prevailing crowded conditions of our poor sections of Panama City and the habit of keeping cats close to them, the need of this study is quite apparent.

All the cats examined were of the species *Felis catus* L.; 34 were male and 62 were female. The stomach and the small and large intestines were removed, opened separately and examined macroscopically for adult parasites which might be present. The walls and contents of the stomach, of the small intestine, leading from the pylorus, and of the large intestine, starting at the appendix, were studied microscopically with the dissecting microscope to determine the incidence of parasites, such as *Oncicola canis, Ancylostoma caninum,* and others. The contents of the appendix were examined for adult parasites, such as *Trichuris vulpis.* Finally two smears were made, and the scrapings of the wall and the contents of the first duodenal section and of the cecum were studied microscopically to determine the incidence of ova, larvae and microscopic parasites. The liver was always examined for flukes.

Specific diagnosis was made by the findings of both eggs and adult specimens in all cases. Of 96 cats examined, helminth parasites were present in 93. Of these 11 were found to harbor only one species; 27 were hosts for two species; 25 for three species; 11 for four species; 13 for five species; 4 for six species; and only 2 were found to harbor seven species. The highest incidence of infection was by *Ancylostoma caninum* 84.5%, followed by *Strongyloides stercoralis* 47.0%, *Toxocara canis* 35.5%, *Physaloptera praeputialis* 27.8%, *Taenia taeniaeformis* 21.1%, *Trichuris vulpis* 20.1%, *Dipylidium caninum* 18.2%, *Diphyllobothrium mansoni* 11.5%, *Oncicola canis* 2.9%, *Physaloptera canis* 1.9%.—C. Calero M., P. Ortiz O. and L. De Souza, *Medical Department, Panama Hospital and The Gorgas Memorial Laboratory.*

OBSERVATIONS ON THE MIGRATORY ACTIVITY OF THE LARVAE OF *TOXASCARIS TRANSFUGA* (RUD. 1819) BAYLIS & DAUBNEY 1922

The writer wishes to make a correction relating to an abstract (J. Parasit. 36: suppl. p. 29) where it was stated that *Ascaris columnaris* had been collected from the black bear, the

marten and the fisher. The nematodes collected from the black bear (*Euarctos a. americanus*) were *Toxascaris transfuga*, and those collected from the fisher and marten have subsequently been found to differ from *A. columnaris*, although the life history appears to be the same. They may be *A. mustelarum* Rud. 1819. The measurements given should be disregarded. A report on the morphology and larval migrations of the ascarid from the fisher will be published shortly. Infection experiments in white mice with the eggs of *T. transfuga* have shown that some of the larvae remain in the wall of the intestine, particularly the rectum and caecum, where they become enclosed in a white nodule in which they remain for many weeks. Others migrate through the liver and lungs and make their way back into the intestine in a manner similar to the larvae of *A. lumbricoides*. Many of the larvae of *T. transfuga* are distributed to the various tissues of the body where they become encapsulated but remain alive for at least ten weeks. The encapsulated larvae are particularly evident beneath the skin of the dorsal and neck regions of the mouse.—J. F. A. Sprent, *Ontario Research Foundation, Toronto, Can.*

LABORATORY METHODS FOR THE EXAMINATION OF MICE FOR OXYURIDS

In connection with the use of oxyurid-infected mice for chemotherapeutic studies, it is desirable to be able to determine infected animals in advance of treatment. By means of the Otto, Hewitt and Strahan (1941, Amer. Jour. Hyg. 33(1): Sec. D: 32–37) modification of the Faust zinc sulfate flotation technique, we found it possible to detect infected animals with a fair degree of accuracy. Mice were isolated in beakers to collect fecal pellets; one or more fecal pellets were put into a shell vial with a small amount of zinc sulfate solution, sp. gr. 1.18; feces were thoroughly emulsified with the solution; more zinc sulfate solution was added until the meniscus was rounded over the edge of the vial; a square coverglass was placed on this and allowed to stand for 10–20 minutes; the coverglass was then removed and examined under low power (100×). Although the first examinations were made on 1–6 pellets, the method was finally standardized using 5–6 pellets and allowing the preparation to stand 15 minutes before examination. Using this technique, eggs of *Aspiculuris* and *Syphacia* and larvae as small as 54 μ in length were floated to the surface.

In attempting to determine the presence of worms at autopsy, it was found very difficult to separate them from the fecal debris, especially in cases of light infection. However, by opening the cecum or large intestine in zinc sulfate solution (sp. gr. 1.18, as for the stool examination) instead of in water or saline, adult worms, larvae as small as 154 μ in length and occasionally eggs were floated to the surface almost immediately and were easily picked out under the dissecting microscope at a magnification of 12×.

At autopsy both the cecum and large intestine were removed and examined separately.

	Stool examination		Autopsy	
	+	−	+	−
Same day	29	1	30	0
	1	3	0	4
Autopsy 2–3 days later	36	0	35	1
Autopsy 4 days later	7	0	6	1
Autopsy 6–7 days later	7	0	7	0
Autopsy 11 days later	6	0	6	0

In addition, 95 test animals positive by stool examination before treatment were positive on autopsy 2–3 days later, 17 positive before treatment were positive at autopsy 6–7 days later, and 10 positive before treatment were positive on autopsy 11 days later.

By the use of these methods, we found a very high correlation between results of stool and autopsy examinations. However, the number of eggs or larvae found on stool examination gave no indication of the number of worms present, as shown at autopsy.—K. L. Hussey and N. E. Alger, *School of Public Health, Columbia University.*

RECORD OF THE LARVAL TREMATODE *HIMASTHLA QUISSETENSIS* (MILLER AND NORTHUP, 1926) STUNKARD, 1934 IN THE CLAM, *MYA ARENARIA*

Stunkard (1938; Biol. Bull. 75: 145–164) experimentally determined the life cycle of *Cercaria quissetensis* (Miller and Northup, 1926) and referred it to the echinostome genus *Hi-*

masthla. He reported encystment in the mantle, gills, and foot of six species of bivalve mollusks experimentally infected. Gulls of the genus *Larus* were reported as final hosts.

The natural occurrence and frequency of the metacercariae in a bivalve intermediate host have not been reported. Observations made during the summer and fall of 1950 in Essex County, Massachusetts indicate that the soft clam, *Mya arenaria,* is an important vector in the transmission of this parasite. Cercarial producing specimens of the mud snail, *Nassa obsoleta,* were readily collected and experimental infections of *Mya* were accomplished for comparative identification. Samples of *Mya* from Merrimack Bay, Plum Island Sound, and Annisquam River show a high incidence of infection ranging from 43 to 100 per cent. Among these samples, the degree of infection ranges from 1 to 99 larvae per clam with larger clams showing a higher degree and frequency of infection; frequency exceeds fifty per cent when mean size of sample exceeds 50 mm. In natural infections, the predominant foci are the palps and gills; few metacercariae were observed in other tissues.

Evidence of widespread range and frequency of these larvae in *Mya* was found in a sample lot of fifty specimens from the region of St. Andrews, N.B., Canada. Of these, forty-five, or ninety per cent, were infected with the larval parasite.

These observations and a consideration of the coincident habitat suggest that *Mya arenaria* is an important vector of *H. quissetensis* throughout its mutual range with *Nassa obsoleta.*—Joseph R. Uzmann, *U. S. Fish and Wildlife Service, Newburyport, Massachusetts.*

NEW RECORDS OF TEXAS SIPHONAPTERA

Among the ectoparasites taken on a recent plague survey by one of us (B.G.H.) on the Whittenburg Ranch, near Stinnett, Hutchinson County, Texas, November 19–22, 1950, were 2 species of fleas not previously recorded from the state either by Eads and Menzies (1949, *A Preliminary List of the Siphonaptera of Texas,* Tex. Jour. Sci., 1: 33–39) or by Eads (1950, *The Fleas of Texas,* Tex. State Dept. of Health Bul., pp. 1–85). The species are *Epitedia wenmanni* and *Stenistomera alpina.* The preferred hosts of the rather rare *S. alpina* are pack rats. This flea has been recorded from California by Augustson (1941, Bull. S. Calif. Acad. of Sci., 40: 138) and from Arizona, Colorado, Montana, New Mexico, Utah and Wyoming by Good (1942, Proc. Ent. Soc. Wash., 44: 131–139). *E. wenmanni* parasitizes a variety of rodents and is widely distributed in North America. Previously, the most southerly recorded point known to us has been Lake Burford, New Mexico (Chapin, 1919, Bull. Brook. Ent. Soc. 14: 50, reported as *Neopsylla similis*). The following ectoparasites including fleas, ticks, and lice were taken: *ex* 29 pack rats, *Neotoma micropus*—4 *Epitedia wenmanni,* 5 *Stenistomera alpina,* 62 *Orchopeas sexdentatus,* 3 *Meringis parkeri,* 2 *Megarthroglossus divisus bisetis,* 27 *Ixodes woodi,* a tick, and 10 *Neohaematopinus neotomae,* a louse; *ex* 3 prairie dogs, *Cynomys ludovicianus*—5 *Opisocrostis hirsutus* and 3 *Neohaematopinus marmotae,* a louse; *ex* 3 deer mice, *Peromyscus maniculatus*—2 *Epitedia wenmanni* and 1 *Monopsyllus wagneri ophidius;* and *ex* 1 kangaroo rat, *Dipodomys ordii*—3 *Epitedia wenmanni* and 2 *Thrassis campestris.*—B. G. Hightower and R. B. Eads, *State Department of Health, Austin Texas.*

A NOTE ON THE JAVELINA FLEA, *JUXTAPULEX PORCINUS*

The following Texas records of the flea, *Juxtapulex porcinus,* which normally parasitizes the javelina or wild pig, are of interest since they are exceptions to what has been considered to be an unusually well developed host specificity: 2 *ex* bobcat, *Lynx rufus,* March 29, 1950, Uvalde Co.; 73 *ex L. rufus,* Aug. 26, 1950, Zavala Co.; 16 *ex L. rufus,* Oct. 20, 1950, Zavala Co.; 8 *ex L. rufus,* Nov. 3, 1950, Zavala Co.; 6 *ex* coyote, *Canis latrans,* Oct. 20, 1950, Zavala Co.; 2 *ex* dog, *C. familiaris,* Oct. 27, 1950, Zavala Co. and 1 *ex* skunk, *Mephitis mephitis,* Nov. 3, 1950, Zavala Co. All of these collections were made by C. W. Johnson and O. L. Walker during Q fever studies which are being supported, in part, by the Research Grants Division, U. S. Public Health Service.

Six *Juxtapulex porcinus* were taken from a deer, *Odocoileus virginianus,* during an ectoparasite survey in Kleberg Co., Jan. 9–12, 1951, by B. G. Hightower of this Department.

Two additional *J. porcinus* from hosts other than the javelina have been seen through the courtesy of James A. Deer, Entomology Department, Texas A & M College: 1 *ex* bobcat, May 1, 1950, Bee Co., coll. J. P. Forgason and 1 *ex* red wolf, *Canis niger,* Sept. 28, 1950, Bee Co., coll. J. P. Forgason.—Richard B. Eads, *State Department of Health, Austin, Texas.*

TRYPANOSOMES—A NEW LOCALE

On August 13, 1949, two (2) adult *Triatoma longipes* were secured in Tempe, Maricopa County, Arizona, from a woodpile located in a residential district of the lower income group. They were macerated on a slide, stained with Wright's stain, and examined microscopically.

One proved to be infected with crithidia and leptomonad stages of *Trypanosoma cruzi Arizona;* the other was negative.

On August 27, 1949, two more adult *Triatoma longipes* were secured from the same locale. These were examined by aspiration of the hind-gut with a syringe and #25 needle, the contents smeared on a slide, stained with Wright's stain, and examined microscopically. In both insects, crithidial and leishmanial stages were demonstrated but differed slightly in appearance from those found in the *Triatoma* of August 13. The balance of the aspirated fluid was injected intraperitoneally into a white rat. Blood smears taken daily for fourteen days, then semi-weekly for three weeks, stained with Wright's stain, and examined microscopically, proved negative for trypanosomes.—JOHN GILLILAND, AND LUCRECE B. DOWELL, *Dowell Laboratories, Tempe, Ariz.*

THE OCCURRENCE OF *GNATHOSOMA SPINIGERUM* (NEMATODA) IN A 33-DAY OLD PUP

In the course of necropsies of young dogs, a 33-day old native pup yielded one semimature *Gnathostoma spinigerum,* penetrating the wall of the stomach together with one ascarid, *Toxocara canis* and ten hookworms *Ancylostoma caninum* in the small intestine. The gnathostoma worm was more outside than inside of the stomach wall. Around the point of penetration on the serosa slight hemorrhages were noted. The parasite measured 10 × 1.33 mm.; globular cephalic bulb 0.66 × 0.40 mm. covered with eight transverse rows of singly pointed hooks, 0.030 × 0.015 mm. Mouth with two lateral trilobed lips, each bears two submedian papillae near the base; middle lobe almost twice as large as lateral, its margin more or less flat while lateral lobes rounded. Oesophagus 2.2 mm.; intestine 7.0 mm. Cuticle transversely annulated; anterior two-thirds of the body bears spines; anterior spines to the middle denticulate with 3 to 4 pointed processes, succeeding 2 to 1 points, towards the end spines being sparsely distributed and simple. Vulva opens 5 mm. from the posterior end; anus terminal.

Africa, Refuerzo and Garcia (1936, Philip. J. Sci., 61: 221–225) recorded 116-day-old male and female *Gnathostoma spinigerum* in an experimental cat to be 12 × 0.612 mm. and 14 × 0.75 mm. with 8 and 11 transverse cephalic hooklets respectively. Prommas and Daengsvang (1937, J. Parasit., 27: 115–116) reported 198 and 223 days prepatent of the infection in two experimental cats. Since the present specimen aproached the size and the number of hooklets of the gnathostomes cited above with prepatent periods of 198 to 223 days, together with the site the nematode was found, being more outside than inside of the stomach wall, it appears that the subject in question could not have acquired the infection post-natally. The age of the host (33 days) suggests that its diet must have been mainly the milk of the dam. These circumstances tend to point to infection of the host *in-utero.* Lastly, as far as the author is aware this constitutes the first report on the natural occurrence of the worm in a juvenile host.—L. M. YUTUC, *College of Veterinary Medicine, University of the Philippines, Quezon City.*

TREATMENT OF MONKEY AMEBIASIS WITH TERRAMYCIN

Among the antibiotics which have been used in treatment of amebiasis are penicillin, streptomycin, neomycin, polymyxin B, chloromycetin, bacitracin and aureomycin. Recently, a new antibiotic, terramycin, attracted attention of the investigators.

Terramycin has been used both *in vitro* and *in vivo* experiments. Hansen (1950, personal communication) found it inhibitory to growth of *Endamoeba histolytica* in dilutions of 1 : 100,000. Hobby (1950, Proc. Soc. Exp. Biol. & Med. 73: 503–511) observed antimicrobial activity of terramycin in mice. Herrell, Heilman, Wellman and Bartholomew (1950, Proc. Staff Meet. Mayo Clin. 25: 183–196) studied pharmacology of terramycin in man. The publication of Most and Van Assenfelt (1950, Ann. N. Y. Academy of Science 53: 427–428) on treatment of human amebiasis with terramycin indicated the value of the new drug in this disease.

This report presents the results of observations on the action of terramycin on *E. histolytica* occurring naturally in macaques.

The monkeys selected for study were *Macacus rhesus* and *Macacus philippinensis.* The majority of the animals had diarrheic or semisolid stools before therapy and were in poor health generally. Their original weights varied from 1.5 to 4.0 kg. The animals were segregated in individual cages which were cleaned and scrubbed daily with lysol. The excreta dropped through false cage bottoms beyond the reach of the animal. Stool specimens were obtained in the morning on clean paper towels placed on receiving pans under the wire floors. Fecal smears were made promptly within 30 minutes after passage of the stools.

The diet of the monkeys consisted of cooked beans enriched by powdered milk, dry yeast and liver fraction – 1 (Wilson Laboratories). In addition they received oranges daily and dog biscuits 2 or 3 times a week. The animals were irradiated with ultra-violet light for 1 to 2 hours daily.

The fecal smears were stained with iron-hematoxylin using Heidenhain's method. The stools of the monkeys were searched for parasites every 2 weeks for 5 consecutive days.

Nine monkeys were treated with terramycin each receiving from 50 to 200 mgm./kg. of the drug (as terramycin hydrochloride) by mouth daily for 8 to 10 days. The results are presented in the accompanying table. After terramycin administration *E. histolytica* was absent from

TABLE 1.—*Oral administration of terramycin hydrochloride to macaques naturally infected with* Endamoeba histolytica

Macacus rhesus & *philippinensis*	Daily dose of terramycin in mgm./kg. body weight	Total dose of terramycin over 8 to 10 days in gm.	*Endamoeba histolytica* in stools after treatment
T	50	1.75	Absent after 12 weeks
K	50	1.75	" " " "
A	100	2.50	" " " "
M	100	2.25	Recurrence in 11 weeks
S	100	2.60	Recurrence in 9 weeks
R	100	2.73	Recurrence in 5 weeks
H	200	4.25	Absent after 12 weeks
J	200	4.00	" " " "
V	200	8.20	" " " "

the stools of six monkeys (T, K, A, H, J, V) at the end of 12 weeks. In three monkeys (M, S and R) the parasite recurred in 11, 9 and 5 weeks, respectively.

It was noted that within 48 hours after administration of the drug the stools of the animals changed from liquid or semisolid to formed and became very bulky and mushy. The animals improved in health, developed better appetite and several gained weight.

The laboratory studies indicated no damage to the liver or kidney, as shown by bromsulfalein and blood urea tests. Blood counts remained normal and electrocardiograms showed no significant change after the therapy.

Supported in part, by the National Institute of Health, Bethesda, Md., and Chas. Pfizer and Co., Brooklyn, N. Y.—ARSENY K. HRENOFF, *University of California School of Medicine, San Francisco, California.*

A NOTE ON *MONILIFORMIS* IN SOUTHERN CALIFORNIA RODENTS

Three immature acanthocephalans of the genus *Moniliformis* were taken from two adult southern grasshopper mice, *Onychomys torridus,* trapped three miles southwest of Victorville, San Bernardino County, California, February 8, 1951. The worms had the following measurements: Body length, 3.1–8.4 mm.; length of proboscis, 271–450 μ; maximum diameter of proboscis, 66–85 μ; number of hooks, 10 rows of 9 each or 12 rows of 8 each; size of hooks, 10.2–17.2 μ; length of proboscis sac, 342–395 μ; length of lemnisci, 0.9–1.78 mm.; width of lemnisci, 20–25 μ. On the basis of measurements other than the body length and the size of the lemnisci the specimens appear to be identical with *Moniliformis clarki* (Ward) as described by Chandler (1947, *J. Parasit.,* 33: 278–281).—DAVID J. DORAN AND CLARK P. READ, *Department of Zoology, University of California, Los Angeles.*

DEVELOPMENT OF CALIFORNIA *TRYPANOSOMA CRUZI* IN THE BAT BEDBUG

During the summer of 1950, numerous bat bedbugs, *Cimex pilosellus,* were collected from the Pacific pallid bat, *Antrozous pallidus pacificus,* at the San Joaquin Experimental Range, O'Neals, California. Western cone-nosed bugs, *Triatoma protracta,* infected with *Trypanosoma cruzi* were brought from O'Neals to the City College laboratory and the parasites inoculated into a white mouse, experiment 142. Laboratory raised *Cimex pilosellus* were fed upon experiment 142 when many trypanosomes were circulating in its blood. Ten days later one small *Cimex* was crushed and the remains examined in a drop of sodium citrate solution. Numerous developmental stages of *Trypanosoma cruzi* were seen in the gut.

One month later, several *Cimex* were noted dead in the plastic box cage. One of these bugs, possibly 4th instar, was dark brown in color, soft and pliable as if filled with a viscous liquid. Examination of a small sample of this liquid exuding from the abdomen revealed numerous parasites. The dead bedbug resembled in physical state previous specimens of infected *Triatoma* which had died from body cavity invasions. Undoubtedly, the parasites infecting this bedbug had also penetrated the body cavity and were developing rapidly as previously observed in *Triatoma* (Wood, 1942, Am. Jour. Trop. Med. *22:* 613–620). Four warmed glass slides were used for smears from this bug. The bedbug was transferred to a drop of mouse blood, spread apart with dissecting needles, stirred quickly and removed. The drop was smeared with another slide after

the technique of Lamy and Bonnel (1949, Trop. Dis. Bull. *47:* 165) for cultures. Slides number 2 and 3 were impression drops from the pieces of bedbug and slide 4 was made from the smeared remains of this insect.

All slides revealed developmental forms of *Trypanosoma cruzi,* especially leishmaniform stages of the parasite. Many transition form parasites from leishmaniform to trypanoform stages were found suggesting the indirect developmental cycle recently described by the writer from mouse muscle (Wood, 1951, Am. Jour. Trop. Med. *31:* 1–11). However, one important difference noted here was the tendency for leishmaniform parasites to produce crithidiform or trypanoform stages by elongation of the parasites' body along the growing axis of the flagellum, after the flagellum half encircles the leishmaniform stage to produce the transition form. There are many examples of such elongating organisms producing large crithidiform parasites typical of the developmental forms of *Trypanosoma cruzi* in the invertebrate. In addition, there are rounded leishmaniform stages with flagella completely encircling their cell body and numerous untwisted trypanoform parasites illustrating the indirect developmental cycle described recently.

This bedbug material resembles both invertebrate development and culture forms as noted previously in *Triatoma rubida uhleri.* The leishmaniform parasites outnumber all other stages showing many dividing forms. Size of these cells from the bedbug as judged by comparison with the white mouse red blood corpuscles ranges from 3 to 8 μ. The cytoplasm of the dividing parasites is deeply basophilic and vacuolated after staining with Jenner-Giemsa. The basophilia gradually diminishes as the organisms mature to the trypanoform parasite and the cytoplasmic vacuoles disappear.

The remains of the dead bedbug mentioned above was placed in a drop of sodium citrate with the dissected gut and body cavity contents of another infected live bedbug. This material was introduced intramuscularly into the right gastrocnemius of a male, 27.5 gram *Mus musculus,* experiment 155. On the 21st and 23rd days after inoculation, one and two trypanosomes, respectively, were observed in tail samples under an 18 mm. coverglass. Six laboratory-raised 3rd instar *Triatoma protracta* nymphs fed to capacity on experiment 155 on the 21st day. On the 15th day after feeding, all of these bugs showed developmental forms of trypanosomes similar to those of the bugs from which experiment 142 was infected.

The writer thanks the California Forest and Range Experiment Station and the Division of Zoology at Davis, University of California for use of facilities. This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.—SHERWIN F. WOOD, *Life Sciences Department, Los Angeles City College, Los Angeles 29, California.*